



ABSTRACT BOOK

Edited by
M Akhtar
MM Awais

1st INTERNATIONAL CONFERENCE ON ZONOSSES-2014

October 16-17, 2014

Organized by:
**Faculty of Veterinary Sciences,
Bahauddin Zakariya University,
Multan-Pakistan**



MULTAN, THE LAND OF SAINTS AND TRADITIONS



Sponsored by Higher Education Commission, Govt. of Pakistan

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Mian Muhammad Awais

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Edited by Masood Akhtar and Mian Muhammad Awais
Faculty of Veterinary Sciences,
Bahauddin Zakariya University, Multan Pakistan

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MESSAGE FROM PATRON-IN-CHIEF



It gives me immense pleasure to extend my heartiest congratulations to the Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan, Pakistan for arranging this marvelous event of 1st International Conference on Zoonoses-2014. Humans have a multifaceted relationship with animals and use them for a variety of purposes from time immemorial. Humans have used animals as a source of food, tools, clothing and draught purpose for millennia. Apart from this, humans have also developed strong emotional bonds with some animal species as companions to share their loneliness. Due to this reason, animals have remained a source of inspiration for humans. Beside all these facts, with the passage of time, it was revealed that animals may prove to be a serious threat to human populations for the transmission of infectious pathogens, more accurately, zoonotic pathogens, having the potential to be transmitted from animals to human beings. Zoonosis is one of the most important and emerging issues of public health concern. During last two decades, world has badly struck with zoonotic infections like Avian Influenza, Severe Acute Respiratory Syndrome, Swine flu, Mad Cow disease with considerably high rates of morbidities and mortalities among the human population. Moreover, zoonotic pathogens are also a big hurdle in international trade of animals and their products and have negative impact on the economy of country. This may also be a major cause of social isolation of a country from rest of the world. Realizing the importance of this important issue, the Faculty of Veterinary Sciences has arranged this event to bring together the professionals under one roof to cope with the problem which is highly appreciable. This conference will provide a forum to the veterinary and medical professionals to interact with each to overcome the zoonotic issues for the betterment of humanity. I heartily welcome the distinguished foreign and national delegates/guests at this conference being held at Bahauddin Zakariya University, Multan and hope it will serve as a catalyst to interact and develop the strategies to safeguard the public from zoonotic diseases.



(Prof. Dr. Syed Khawaja Alqama)
Vice Chancellor
Bahauddin Zakariya University,
Multan-Pakistan



MESSAGE FROM CHIEF ORGANIZER

Zoonotic pathogens represent serious threat to human health and generate a great covenant of public health concern and scientific interest in both veterinary and medical sectors. According to a recent report, 75% of the emerging diseases originate from domestic or wild animals. Zoonotic agents include bacteria, viruses, fungi, parasites and/or other agents. The animal reservoirs of zoonotic pathogens can be domestic animals, wildlife, pets or peri-domestic wildlife. Concern is grown towards zoonotic diseases because of multiple factors like their unpredictable nature, their ability to emerge anywhere and spread rapidly around the globe and their major economic impact on industries. Unfortunately, most of the zoonotic diseases are neglected, having received a lack of attention from researchers, policy makers and funders for many years. In developing countries like Pakistan, the status of public health is not up to the mark. So, need of the day is to make strenuous efforts to increase our knowledge and understanding of current and probable future prospects of zoonotic diseases. In order to highlight the importance of zoonotic diseases and to promote greater multi-disciplinary input; the Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan, Pakistan has arranged this 1st International Conference on Zoonoses-2014. The researchers and experts in this conference are expected to explore the reasons and factors driving the emergence of zoonotic diseases to prominence and to identify more efficient strategies and research programs that need to be developed for better understanding and control of emerging zoonotic infections and their public health impact. This conference will provide the professionals to get advanced knowledge on detection and control of zoonotic diseases from invited speakers who are authorities in their area of research zoonotic diseases. This will also enable them to develop and promote more effective disease control strategies and to improve animal and human health care systems the concept of “One Health”.

I do hope that participants will find this event a valuable addition in their existing scientific knowledge and wish them a great success in their future endeavors.

A handwritten signature in black ink, appearing to read 'Masood Akhtar'.

(Prof. Dr. Masood Akhtar)
Dean, Faculty of Veterinary Sciences,
Bahauddin Zakariya University,
Multan-Pakistan

ICZ-2014-001

ZOONOSES: NEW AND EXISTING THREATS TO PUBLIC HEALTH FROM ANIMALS

Alasdair JC Cook

Department of Veterinary Epidemiology & Public Health, School of Veterinary Medicine, Faculty of Health & Medical Sciences, Duke of Kent Building, University of Surrey, Guildford, UK

The risk to human health from diseases that occur in animals has been recognised from ancient times, long before the nature of infectious agents was understood. There are cultural behaviours with respect to attitudes to animals and their products that are related to the perceived risk from these zoonotic diseases. Today, there are economic, social and environmental pressures that increase the human burden of zoonotic disease, including globalisation of the trade in animals and animal products, intensification of animal production, population growth, encroachment on native ecosystems and climate change. These are resulting in altered risks associated with recognised zoonoses and also in the emergence of new diseases. Zoonotic diseases have caused billions of pounds of economic damage already in this Century and result in perhaps a billion cases of disease and millions of deaths every year. This paper will address the risks associated with zoonoses and consider the role of the veterinary profession within the One Health concept for the early detection and control of these conditions

Keywords: *zoonoses; threats; public health; animals*

ICZ-2014-002

AN UNUSUAL SICKNESS IN EQUINES WITH ZONOTIC IMPLICATIONS

Muhammad Ashraf

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Infectious and contagious diseases are not un-common in animals. Glanders is a contagious and fatal disease of horses, donkeys, and mules. It is caused by infection with the bacterium *Burkholderia mallei* (the name recently changed from *Pseudomonas mallei* and was previously classified as *Pfeifferella*, *Loefflerella*, *Malleomyces* or *Actinobacillus*). The disease causes nodules and ulcerations in the upper respiratory tract and lungs. A skin form also occurs, known as 'farcy'. Control of Glanders requires testing of suspect clinical cases, screening of apparently normal equids and elimination of positive reactors. Outbreaks of the disease may occur in members of the cat family living in the wild or in zoological gardens. Glanders in the acute form occurs most frequently in donkeys and mules, which run a high fever and exhibit respiratory signs (swollen nostrils, dyspnoea, and pneumonia); death occurs within a few days. In horses, Glanders generally runs a more chronic course and they may survive for several years. Chronic and sub-clinical cases are dangerous sources of infection.

Keywords: *glanders; equines; zoonosis*

ICZ-2014-003

**UPDATES ON BOVINE TUBERCULOSIS IN CATTLE AND BUFFALOES,
SPECULATED FUTURE OF DISEASE AND CONTROL PROGRAM FOR
PAKISTAN**

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The published data reveal varied prevalence of disease in different regions of Pakistan. The prevalence is lower than 10% at most places, higher than that but lower than 20% at some places. Most of the studies are carried out by skin testing and very few studies involve the abattoirs which are mainly restricted to Lahore and Faisalabad. There is no regular screening of animals in the country except at public owned livestock experiment stations. A report published in 1969 indicates prevalence of 6.72% in dairy animals in Faisalabad. In 1972, a prevalence of 8.3% in Sahiwal cattle and 2.9% in buffaloes was reported. In early 1970s, 0.53% prevalence in Rohri area and 5.31% in Quetta has been reported in buffaloes. In 1979, an overall prevalence of 9.1% was reported. A report published in 1989 indicated 7.3% prevalence in cattle and buffaloes slaughtered at Lahore abattoir. In 1992, an abattoir based study in Lahore reported 7% prevalence in buffaloes. In 2001, a prevalence of 1.76% in buffaloes and 5.1% in cattle was observed in the Faisalabad area. In the same year in Lahore area a prevalence of 1.3% was observed in cattle. In year 2003, a report indicated 6.91% prevalence in buffaloes and 8.64% in cattle in the Lahore area. In 2006, the prevalence in buffaloes at two experimental livestock farms in Punjab was 2.45 and 8.48%, respectively. In 2009, a prevalence of 2.2% in buffaloes around two cities of Punjab was reported. In 2010, 3% of buffaloes were found positive with herd prevalence of 14%. In 2011, 7.6% prevalence was recorded in cattle with 100% herd prevalence, although the animal level prevalence varied from 2.0 to 19.3% of these farms. In 2012, 11.3% prevalence was recorded in buffaloes with 86% herd prevalence. The future of disease looks to be graved as there is no knowledge about disease to the local farmer and animals are being purchased without testing. As the dairy farming is rapidly turning into one of the profitable future industry with farms are rapidly expanding so the disease situation is speculated to become worse. Therefore, there is need to have a control program in place to keep the disease in animals to the minimum which require involvement of the Government, stake holders and community in large. The control program can be multifaceted which also require necessary

Keywords: tuberculosis; bovines; control program

ICZ-2014-004

ZOONOTIC POTENTIAL OF VARIOUS *BABESIA* SPECIES AND STATUS OF EQUINE PIROPLASMOSIS IN SAARC COUNTRIES

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Babesiosis, a tick transmitted haemoprotzoan disease in different sp. of animals causes heavy economic losses due to high mortality, decreased production and lowered working capacity of the affected animals in tropical and sub-tropical parts of the world including SAARC countries where ticks are in abundance. For more than half a century, various Babesia sp. were considered to be extremely host specific. However, in recent years many human cases of babesiosis have been reported from various countries. Equine piroplasmosis (EP), also a tick transmitted haemoprotzoan disease caused by Theileria (Babesia) equi and/or Babesia caballi is globally distributed including SAARC countries with particular reference to India, Pakistan and Afghanistan. This disease condition poses a serious threat in international movement of affected horses. The infectivity rate of T. equi in the salivary glands of Hylomma anatolicum anatolicum ticks has been assessed, which provided an important insight into transmission potential of infected ticks and hence designing better control strategies. The conventional serotests available were not sufficient for sensitive and specific diagnosis of the latently infected equids. Hence, new improved diagnostic methods have been designed for accurate and rapid diagnosis. Demonstration of parasites in the blood collected from a carrier animal by blood smear examination or by in vitro cultivation technique is an absolute and undoubtable method of confirmation. In vitro cultivation of T. equi and B. caballi by MASP technique is the indispensable methodology for demonstration of live parasites and only a few laboratories in the world (including India) have this facility. Recently, geographically conserved genes in T. equi genome (ema1 and ema2) were exploited in PCR (and nested PCR), DNA probe and ELISA. These techniques proved to be an efficient tool in specific diagnosis, but are limited to well equip laboratories. A remarkable improvement in ELISA has been made and problems associated with native antigen were overcome by production of recombinant antigen. cELISA has proved to be highly sensitive and specific and recently been prescribed by OIE as a test for international trade. In Pakistan, the overall sero-prevalence of EP (by cELISA) was found to be 41.2% for T. equi and 21.6% for B. caballi. Recently, recombinant antigen based _{EMA-2}ELISA

has been developed and used for sero-surveillance on Indian equine population. Analysis of 5651 equine serum samples ? collected during 2007 to 2012 from 12 states of India representing eight agro-climatic zones ? by _{EMA-2}ELISA revealed 32.65% sero-prevalence of T. equi in India. Latex agglutination test (LAT) and immunochromatographic test (ICT) are the newly developed tests based on specific recombinant antigen and are much faster than the other available diagnostic tests/assays; further validation may, however, be required. Theileria equi organism and its metabolites inflict oxidative damage on red blood cells (RBCs) which was evident by increased RBCs membrane protein, phospholipids, plasma MDA levels in infected donkeys. This implies that oxidative damage ameliorating drugs may be beneficial for treatment of this disease condition. Artemether (qinghaosu) derivatives, was tested in combination with buparvaquone on T. equi experimentally infected donkeys. Results revealed that arteether+buparvaquone combination could be a better choice than imidocarb for treating T. equi infection, as less deleterious effects on the liver function test were observed in this drug combination group. No systematic sero-prevalence of this disease condition has been undertaken in SAARC countries and there is an urgent need to take up this activity on priority, so as to make collective efforts for identifying the endemic areas and plan systematic control strategies.

Keywords: *Babesia; equine piroplasmosis; SAARC countries*

ICZ-2014-005

TOXOPLASMA GONDII ZOOSES: STATUS, CHALLENGES AND FUTURE DIRECTIONS

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Toxoplasma gondii is one of the most successful, ubiquitous, water and food borne, zoonotic, apicomplexan, obligate, intracellular, eukaryotic protozoan pathogen. It is the only species in the genus Toxoplasma that parasitizes and infects virtually any warm blooded animal including those living exclusively in the sea. T. gondii can be transmitted orally among intermediate hosts and produces infection without cycling through its definitive feline host. This provides it the potential for clonal spread among wide range of intermediate hosts. Up to one third of the human population in the world is chronically infected with this parasite and more than 60 million people in United States alone are believed to be infected. It has emerged as a major opportunistic pathogen in immunocompromised patients and is also responsible for congenital defects in humans. The prevalence of T. gondii ranges from 15-85% worldwide with the incidence of Toxoplasma infection in France being greater than 90% among adults. Sero-epidemiological studies on isolated islands in the Pacific, Australia and the United States where very few cats are present, have shown a very low seroprevalence of T. gondii, confirming the important role of the cat in the natural transmission of T. gondii. The conventional diagnosis of T. gondii infection usually employs serological tests, bioassays in cats and/or mice, or a combination of the two approaches. In the past two decades, the diagnosis of T. gondii infection by direct detection of parasite-specific DNA in biological samples using molecular methods has gained popularity. Analysis of the data indicates that subpopulation structures in different continents have a few clonal lineages. In Europe and North America, a large number of T. gondii isolates have shown remarkably little diversity and have been classified into one of the three clonal genetic lineages (Types I, II, III). Recent population genetic studies of T. gondii from South America and other geographical regions have revealed high frequency of non type I, II and III genotypes. Limited sampling, based largely on

RFLP markers suggests that African populations of *T. gondii* are similar to those in Europe and North America. In contrast, studies from Asia suggest predominance of genetically mixed strains. We still do not know the complete makeup of parasite populations in Africa, Asia, and other parts of the globe where *T. gondii* is abundant. The study of correlation between the parasite genotypes and the disease manifestation in human toxoplasmosis is an area of considerable interest and needs future investigation. The ability of *T. gondii* to infect a wide range of hosts presents a difficult challenge for the management of the parasite for persons who study, treat or make a research on different aspects of this parasite. The integrated approach combining molecular detection and high resolution genetic characterization will provide maximum information on molecular epidemiology and population genetics in order to address questions regarding the association of disease manifestations with parasite genotypes and virulence. This will help in developing intervention strategy to prevent the spread of *T. gondii* transmission and reduction of toxoplasmosis both in animals and humans

Keywords: *Toxoplasma gondii*; zoonoses; status; control strategies

ICZ-2014-006

SPATIAL ECOLOGY AND EPIDEMIOLOGY OF SOIL-BORNE PATHOGENS OF VETERINARY AND PUBLIC HEALTH SIGNIFICANCE IN AND AROUND VILLAGES OF DISTRICT LAHORE, PAKISTAN

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Besides nutrients and mineral, the soil harbors globally distributed pathogens of both veterinary and public health significance. The implications and consequences are more sever in developing countries particularly Pakistan where limited diagnostic and research capabilities coupled with lack of proper reporting system is present. Here we report the prevalence of selected soil-borne pathogens viz. Bacillus (B.) anthracis, Yersinia (Y.) pestis, Burkholderia (Burkh.) mallei/pseudo malleii, Francisella (F.) tularensis and Coxiella (C.) burnetii in and around the village of district Lahore, Pakistan. Soil samples (n = 145) were collected aseptically from the randomly selected villages (n = 29, 5 sample from each village) along with detailed relevant history. The samples were processed for real time PCR based identification of selected pathogens along with soil chemistry and several risk-factor analysis. Real time PCR detected F. tularensis, B. anthracis (CapB), C. burnetii and Burkh. mallei in 13.10% 9.65%, 4.83% and 1.37% of soil samples, respectively. None of the sample was detected with protective antigen plasmid of B. anthracis and Y. pestis. The prevalence of B. anthracis (CapB), F. tularensis and C.

burnetii was found to be associated with phosphorus, magnesium, copper, chromium, manganese, cobalt, cadmium, sodium, ferrous, calcium, potassium; phosphorous and copper, respectively. With respect to B. anthracis (CapB), a non-significant association of risk-factors was seen. The prevalence of F. tularensis was found to be significantly associated with main road, while for C. burnetii, it was found to be associated with main road, vegetation, canal/drain, animal and human density. The preliminary findings showed that frequency of detection of soil-borne pathogens were more frequent in areas where animals were held prior to auction, transport and slaughter and these locations were along the interstate routes in Lahore. Further studies are needed to ascertain the genetic nature of prevalent strains and appropriate interventions for their subsequent prevention and control

Keywords: spatial ecology; epidemiology; soil borne pathogens; Lahore

ICZ-2014-007

LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY: A POWERFUL INOVATIVE DIAGNOSTIC TOOL FOR THE RAPID MOLECULAR DETECTION OF ZOONOTIC PATHOGENS IN DEVELOPING COUNTRIES

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Conventional diagnostic capabilities (culture and serology) are insufficient to effectively monitor the zoonotic 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 simple and cost effective diagnostic test for the molecular detection of many man & animal diseases, especially in developing countries because no costly equipment like thermal cycler is required to perform the test. The LAMP uses a set of minimum 4 primers that target 6 sites of a conserved gene. The LAMP assay relies on autocycling strand displacement DNA synthesis performed by *Bst* or *Bsm* 2.0 DNA polymerase. Being a very sensitive test the results can be visualized real time by observing 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 favouring point-of-care testing (POCT). The POCT format was originally demonstrated for neglected tropical diseases (NTDs), where access to reliable diagnostic testing is severely limited. Loop-mediated isothermal amplification (LAMP) is an innovative molecular technique that has been validated for point-of-care testing to diagnose many zoonotic pathogens like tuberculosis, brucellosis, zoonotic simian malaria, *Echinococcus granulosus*, zoonotic Chlamydiosis, Toxoplasmosis, Leishmaniasis, Trypanosomiasis, Rabies and Avian Influenza etc. We believe that an efficient disease diagnosis through LAMP will ultimately strengthen the molecular capabilities of developing world to combat with zoonoses and public health issues.

Keywords: *LAMP; molecular diagnosis; zoonotic pathogens; developing countries*

ICZ-2014-008

TETANUS IN EUROPE: A CASE NOT YET CLOSED!

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Tetanus is a sporadic and relatively uncommon infection in EU/EEA countries, caused by the bacterium *Clostridium tetani*. Contamination of wounds with tetanus spores in unimmunised persons can cause an illness with muscular spasms and sometimes death. Tetanus is included in the primary vaccination schedule of all EU countries, and periodic vaccination in adulthood is required to maintain immunity. The latest official numbers available by ECDC (European Centre for Disease Prevention and Control) are referred to 2010. In that year, 130 cases, including 74 confirmed cases meeting the EU case definition, were reported by 12 EU/EEA countries. Austria, Denmark Finland, Germany and Liechtenstein did not report. Italy, Poland, France, Romania, the United Kingdom and Spain accounted for most of the notified cases. Italy accounted for 57 of the 74 confirmed cases reported in 2010. Italy has been continuously reporting the highest number of tetanus cases since 2006, ranging between 53 and 64 cases per year. The overall confirmed case rate remains low at 0.02 per 100 000 population. The highest rate was reported by Italy (0.09 per 100,000). The confirmed case rate of tetanus remains low in the EU. This may be explained by the fact that laboratory confirmation is usually not performed for tetanus and diagnosis is based on clinical presentation. The widespread use of tetanus vaccination in EU/EEA countries also contributes to a low disease rate. The cases reported in the elderly were probably related to lower coverage or waning immunity in this population. The high proportion of women could be explained by different vaccination strategies during their youth, particularly in relation to vaccination on enrolment to mandatory military service for men and occupational vaccination programmes. This emphasises the need to maintain high vaccination rates in workers exposed to biological risk and to implement catch-up/ booster strategies in countries which have a higher rate of disease.

Keywords: *Tetanus; Clostridium tetani; Europe*

ICZ-2014-009

BRUCELLA ABORTUS IN LARGE RUMINANTS IN BANGLADESH

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Brucellosis is considered to be the most widespread zoonosis throughout the world caused by different species of the genus *Brucella*. In animals, brucellosis mainly affects reproduction and fertility, with abortion birth of weak offspring and reduced milk yield. In man, the clinical picture resembles many other febrile diseases, but sacroiliitis and hepato-splenomegaly are the most prominent symptoms. Severe complications are endocarditis and neurological disorders. Prevalence of brucellosis has been widely investigated on the basis of serological test in livestock but no study was devoted to identify the specific *Brucella* species prevalent in Bangladesh. The objective was to determine the prevalent *Brucella* species in cattle and buffaloes in Bangladesh. For these purposes, a total of 799 serum samples of cattle and buffaloes were collected from the different districts of Bangladesh. RBT, SAT, CFT, ELISA were performed according to the procedures described by the manufacturers. The RBT positive sera were re-tested with SAT, CFT, ELISA, and qRT-PCR. For the qRT-PCR, DNA was isolated from 200 µL of seropositive serum using the High Pure PCR Template Preparation Kit according to the manufacturer's instructions. *Brucella* IS711 targeting genus specific qRT-PCR was done according to the established and routine protocol on a light cycler 2.0 instrument. Positive samples were then typed with the *Brucella* IS711 species specific qRT-PCRs for *B. abortus* and *B. melitensis*. Out of total 700 cattle sera and 99 buffalo sera, 37 cattle sera and 7 buffalo sera showed positive reaction to RBT with the overall prevalence of brucellosis 5.29% (95% Confidence Interval (CI): 3.6-6.95) in cattle and 7.07% (95% CI: 2.02-12.12) in buffalo. The genus specific screening by PCR detected *Brucella* DNA in 24 sera where 17 sera of cattle and 7 sera of buffaloes. The species specific IS711 PCR also detected *B. abortus* DNA from the 19 sera samples where 13 sera of cattle and 6 sera of buffaloes. The presence of *Brucella* DNA was found in 4.43 % of the cattle and 7.1 % of the buffaloes investigated. *B. abortus* DNA was found in 1.9% of cattle and 6.1% of the buffaloes in this study. The genus specific screening by PCR detected *Brucella* DNA in 24 sera where 17 sera of cattle and 7 sera of buffaloes. The species specific IS711 PCR also detected *B. abortus* DNA from the 19 sera samples where 13 sera of cattle and 6 sera of buffaloes. The presence of *Brucella* DNA was found in 4.43 % (95% Confidence Interval (CI): 2.91-5.95) of the cattle and 7.1 % (95% CI: 2.04-12.16) of the buffaloes investigated. *B. abortus* DNA was found in 1.9% (95% CI: 0.89-2.9) of cattle and 6.1% (95% CI: 1.38-10.8) of the buffaloes in this study. This is the first proof that *Brucella abortus* is endemic in cattle and buffaloes in Bangladesh. A combination of (real-time) PCR with RBT could be effective for future eradication programmes.

Keywords: brucellosis; cattle; buffalo; Bangladesh; serology; PCR

ICZ-2014-010

GENOMIC SEQUENCE ANALYSIS OF PRION PROTEIN GENE IN DROMEDARY CAMEL BREEDS OF PAKISTAN

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Prion proteins, encoded in higher species, can cause serious neurodegenerative disorder due to transformation of cellular prion protein into its pathological isoform that is transmissible to humans. Camel has played an important role in socio-economic sector of Asia and Africa through survival in life-threatening drought periods, transportation, combat desertification and food security in arid and hot climatic desert region. This has made them superior to other domestic mammals. Pakistan has more than a million heads of dromedary camel (*Camelus dromedarius*). To have an idea about Prion protein gene (PrP) in Pakistani camels we collected blood samples from 10 different camel breeds from different regions of the country. PrP gene specific primers were utilized to execute amplification of coding region. CodonCode Aligner software was used to align and edit the sequences and a final 667 bp fragment was selected for further analyses. PrP sequences from other mammalian species were selected from GenBank NCBI to compare with Pakistani camel and phylogenetic analysis was done using MEGA 6.1 and distance matrix was constructed using Multidimensional Scaling Plot. That was the novel study accomplished on Pakistani camel breeds which reconfirmed the biological classification of mammalian species and highlighted possible factors affecting the prion disease. Analyses showed that Pakistani camels were more closely related to Indian dromedary camel as compared to Wild Bactrian Camel and Alpaca, elucidating evolutionary changes occurred in them. This is the first report on PrP gene in camel breeds of Pakistan and these findings might help future scientists to explore important genetic factors related to PrP through computational means.

Keywords: *prion protein gene; sequence analysis; phylogenetics; MDS plot*

ICZ-2014-011

SEROPREVALENCE OF *TOXOPLASMA GONDII* IN THE BACKYARD CHICKENS OF THE RURAL AREAS OF FAISALABAD, PUNJAB, PAKISTAN

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This study reports the seroprevalence of *Toxoplasma (T.) gondii* in backyard chickens of rural areas of district Faisalabad, Punjab, Pakistan. Backyard chickens (n=300) were selected randomly from five different villages of district Faisalabad, Pakistan. Blood samples were collected randomly and subjected to Latex agglutination test for screening of *T. gondii*. Seropositive chickens were sacrificed to collect blood. Vital organs including heart and brain were also collected for histopathological examination and mouse bioassay. Overall seroprevalence of *T. gondii* was 36.33%. Area- and sex-wise seroprevalence was detected as non-significant (P>0.05). Age-wise analysis showed highest seroprevalence rate (57.14%) in chickens of age group ranging from 1.5-2 years (P=0.00). The chickens kept along with pet cats showed higher seroprevalence (53.89%; 95% CI=0.401, 1.375) as compared to those kept without pet cats. Feeding and watering patterns showed non-significant (P= 0.085; OR=0.643) impact on the seroprevalence of *T. gondii*. In mouse bioassay, toxoplasmosis was reproduced only in 40% of the mice population being infected. Histopathological studies revealed congestion, necrosed areas and inflammatory cells in brain and heart. Findings of the present study concluded that infection of backyard chickens with *T. gondii* is prevalent in Faisalabad, Pakistan which may have significant public health concerns and implications for prevention and control of toxoplasmosis in this area.

Keywords: *Toxoplasma gondii*; seroprevalence; backyard chickens; rural areas; Pakistan

ICZ-2014-012

ISOLATION AND TYPING OF THERMOPHILIC CAMPYLOBACTER FROM FREE RANGE TURKEY FARMS IN GERMANY

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The organic farming practice of meat producing turkeys has been subjected to the strict rules regarding the use of antimicrobial substances on the farms. The free range turkey farms must be provided with organically produced feed, fresh air, sunlight, and the free outside environment. This poses a risk that they possibly get infected with Campylobacter. This study obtained baseline information on the prevalence as well as estimating the genetic characterization of thermophilic Campylobacter as biological hazard in free living organic turkey farms in Germany. Five ecological turkey farms (4 to 8 weeks age), epidemiologically unrelated, were sampled during the rearing cycle. The turkey cecal samples and environmental samples include drinking water and dark beetles in each farm were collected, identified and analyzed for Campylobacter by conventional and molecular methods. The resulted isolates were differentiated and typed by flaA-RFLP analysis. Campylobacters were detected in caecal swabs derived from all 5 turkey farms with prevalence (95% to 100%). Both *C. jejuni* (14.3% to 66.7%) and *C. coli* (33.3% to 85.7%) were detected. Campylobacter was only detected in drinkers only in 2 farms and the internal contents of dark beetles in the 2nd farm. No Campylobacter was isolated from main water tank. flaA-RFLP assay showed that the turkey farm could be harboured more than one genotype reached to 4 in case of *C. coli* and 5 in *C. jejuni*. Moreover, 2 different types could be detected in a single turkey. This study revealed that organic turkey flocks are often contaminated at early age with Campylobacter with high prevalence. Also, water and darkling beetles can play a possible role in the entry of Campylobacter into turkey flocks. The understanding of the relationship between beetle infestation, water contamination and the Campylobacter status of organic turkey flocks should enable progress in further development of biosecurity control measures.

Keywords: *isolation and typing; Campylobacter; turkeys; Germany*

ICZ-2014-013

WHEN AVIAN FLU WILL BECOME HUMAN FLU?: HUMAN ANIMAL INTERFACE OF INFLUENZA

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Influenza virus belonging to orthomyxoviridae family is an important pathogen due to its zoonotic importance in human population. Influenza type A virus infects human, horses, pigs and all avian species. Avian influenza (H₅N₁, H₇N₇ and H₉N₂) virus spreads rapidly among avian species and occasionally it also spreads to human population. So far the people died of avian flu were immunocompromised. However no authenticated report from FAO/WHO is available of its spread from human to human. There are two risks involved to humans from transfer of zoonotic diseases direct transfer from these animals to their human handlers, and human to human resulting from a zoonotic disease infection that has mutated. It is a matter of serious concern for human and veterinary health professionals. The H₉ and H₇ types of avian influenza viruses are getting more importance from zoonotic aspects in Pakistan. The target individuals for this zoonotic disease include farm workers, poultry, butchers, poultry veterinarians and house hold ladies dealing with uncooked meat. Many outbreaks of human influenza have occurred in the past which lead to heavy losses of human life. In Pakistan only few human deaths have been reported in the near past but no pandemic. In the present study these aspects of human animal interface of influenza virus is covered. The last case of avian influenza was reported from Pakistan in 2004 and it was documented by the FAO/OIE. The migratory birds have an important role in the spread of avian influenza. The avian viruses lead to mild disease in human however luckily avian viruses not efficiently transmitted among humans.

Keywords: avian flu; human flu; human-animal interface

ICZ-2014-014

**ACTION PLAN TO PREVENT AND CONTROL THE ZONOTIC INFECTIONS
IN HUMANS INVOLVED IN POULTRY BUSINESS – AVIAN FLU CASE STUDY**

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Infections that can cross species barriers and have ability to infect humans working with animals are termed as zoonotic infections. It is estimated that three fourths of the newly emerging diseases are zoonotic in origin. One of the salient reason for this very high transmission of animal diseases to humans is factory farming especially in case of poultry environment controlled farming and massive hatching of chicks. Rapid increase in human population is another factor for increase in contact time with animals/poultry species, this situation is further distorted when this increased population is under nourished and immunocompromised. Moreover increasing population is demanding more space to accommodate which results in decrease land so the farms which were established away from population are invaded by this increasing population. Influenza A type virus infection can be classified into low pathogenic and high pathogenic infections. High pathogenic influenza virus strains include H₁N₁, H₃N₂ and H₅N₁ etc. this infection can cause a wide variety of symptoms ranging from conjunctivitis to dyspnea, acute respiratory distress which may lead to respiratory failure etc. Sometimes it can show even neurologic symptoms like fits along with abdominal pain, nausea and diarrhea. Diagnosis of highly pathogenic influenza A from B or C type virus is not possible as this virus shows overlapping symptoms and requires laboratory testing. People involved in poultry occupation are on the front line to get infected with HAPI/ bird flu infection these include broiler, layer, breeder and primary breeder farmers, their employees, service crews, technical staff, people involved in processing and further processing of poultry and poultry products, people involved in marketing of live birds (brokers, wholesalers and retailers etc.), hatchery workers and chick sale and delivery staff, including truck drivers, disease control and biosecurity workers, veterinarians and para-veterinary staff and government officials working with destroying of infected flocks to curtail the HAPI infection. Moreover all the people who can get in touch with environment contaminated with HAPI infection directly or indirectly these may include the waster busters, cleaning and disinfection crews also have the risk of HAPI infection as secretions and excretions of infected birds is also a potential hazard. The most effective tool to control bird flu is to prevent it. Prevent, detect and controls are the various steps to control Avian flu infection in poultry and its subsequent transmission in humans. An effective biosecurity program should be in place to prevent avian flu in poultry enterprises.

Keywords: *poultry industry; zoonotic infections; avian flu*

ICZ-2014-015

**CRIMEAN CONGO VIRUS IN PAKISTAN: A CRITICAL ANALYSIS OF
DIAGNOSTICS, TRANSMISSION DYNAMICS AND EPIDEMIC THREATS**

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Crimean Congo hemorrhagic fever (CCHF) is a highly contagious tick-borne zoonotic infection. The disease is transmitted from animals to humans through the bite of infected tick, and from humans to humans through contact with blood, tissues and body fluids of infected person. Pakistan is regarded as an endemic region for CCHF as large number of outbreaks, sporadic and nosocomial cases have been reported and the number of cases increases every season. Most of the cases in Pakistan are reported in Baluchistan and Khyber Pukhtunkhwa due to the dry weather that supports the growth of ticks. Among them majority of the cases are transmitted from Afghanistan because of the cross border trade and communication with Afghanistan. Migratory birds carrying ticks may probably play part in transmission of the virus. While treating suspected CCHF cases, doctors and the first line health care workers are at the highest risk of contracting the disease. The first case of CCHF in Pakistan was reported in 1976 in Holy Family Hospital Rawalpindi. The Health system in Pakistan has not been able to establish at least BSL-3 lab with diagnostic facilities for CCHF. The initial diagnosis is based on signs and symptoms. The samples are sometimes sent to National Health Institute in Islamabad to be dispatched abroad for confirmation of cases. Lack of awareness among the health professionals', unsatisfactory preventive measures and zero diagnostic and research facilities will results in frequent outbreaks and more deaths in Pakistan in the coming years.

Keywords: *Crimean Congo virus; transmission; epidemic threat; Pakistan*

ICZ-2014-016

MOLECULAR EPIDEMIOLOGY OF MYCOBACTERIUM THROUGH INTERFERON GAMMA RELEASE ASSAY AND DIFFERENTIAL PCR IN ANIMAL HOLDERS

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Infections that can cross species barriers and have ability to infect humans working with animals are termed as zoonotic infections. It is estimated that three fourths of the newly emerging diseases are zoonotic in origin. One of the salient reason for this very high transmission of animal diseases to humans is factory farming especially in case of poultry environment controlled farming and massive hatching of chicks. Rapid increase in human population is another factor for increase in contact time with animals/poultry species, this situation is further distorted when this increased population is under nourished and immunocompromised. Moreover increasing population is demanding more space to accommodate which results in decrease land so the farms which were established away from population are invaded by this increasing population. Influenza A type virus infection can be classified into low pathogenic and high pathogenic infections. High pathogenic influenza virus strains include H₅N₁, H₅N₂ and H₅N₃, etc. this infection can cause a wide variety of symptoms ranging from conjunctivitis to dyspnea, acute respiratory distress which may lead to respiratory failure etc. Sometimes it can show even neurologic symptoms like fits along with abdominal pain, nausea and diarrhea. Diagnosis of highly pathogenic influenza A from B or C type virus is not possible as this virus shows overlapping symptoms and requires laboratory testing. People involved in poultry occupation are on the front line to get infected with HAPI/ bird flu infection these include broiler, layer, breeder and primary breeder farmers, their employees, service crews, technical staff, people involved in processing and further processing of poultry and poultry products, people involved in marketing of live birds (brokers, wholesalers and retailers etc.), hatchery workers and chick sale and delivery staff, including truck drivers, disease control and biosecurity workers, veterinarians and para-veterinary staff and government officials working with destroying of infected flocks to curtail the HAPI infection. Moreover all the people who can get in touch with environment contaminated with HAPI infection directly or indirectly these may include the waster busters, cleaning and disinfection crews also have the risk of HAPI infection as secretions and excretions of infected birds is also a potential hazard. The most effective tool to control bird flu is to prevent it. Prevent, detect and controls are the various steps to control Avian flu infection in poultry and its subsequent transmission in humans. An effective biosecurity program should be in place to prevent avian flu in poultry enterprises.

Keywords: *tuberculosis; molecular epidemiology; interferon gamma release assay; animal holders*

ICZ-2014-017

BUFFALO POX VIRUS: AN EMERGING THREAT TO BUFFALOES AND HUMAN

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Buffalo pox virus (BPV) was identified long ago in 1934 in India. Since then frequent outbreaks of buffalo pox virus were reported from buffaloes raising area of the world. It was not a serious concern for human until 1980 when small pox was eradicated from the face of globe and its further vaccination was ceased. Now there is naïve population of human beings who do not have resistant against Poxviridae, so BPV spread from buffalo to people who attend them. In Pakistan, first cases of BPV were observed in Cattle colony Karachi where fine specimens of animals are brought there from throughout the country. The dry animals which acquired BPV infection there are sent back to different cities of Pakistan including Sind, Punjab and Baluchistan and spread infection there. Recently we attend an outbreak of BPV in high milk producing buffaloes raising area of central Punjab. A new buffalo was introduced on a farm comprised of 100 adult and 50 young stocks. Afterward, Buffalo pox lesions were observed in few animals which spread among 70% animals of the herd in few days. The poxes like lesion were observed on the udder, teats, limbs, around the ear, and hindquarters. As a result there was loss in milk production and mastitis due to invasion of secondary bacterial infections that intensified the economical loss. The Scab from affected areas of animals were collected and inoculated into experimental rabbit and also subjected to PCR. The PCR and animal inoculation tests confirmed the presence of BPV in the herd. Beside heavy economic losses on the farm, one serious concern was the milk men who also developed BPV lesion on their hands. Although it is believed that BPV does not transmit from human to human but BPV lesions has also observed among young family members of milkmen who are not directly involved in animal handling. So we suggest further investigation how the virus is transmitted among these individuals.

Keywords: *buffalo pox virus; emerging threat; human beings*

ICZ-2014-018
RABIES SITUATION IN PAKISTAN

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Rabies is fatal viral zoonotic disease theoretically of all warm blooded animals. Rabies is among few zoonotic diseases which has cent percent case fatality rate. Although timely use of available biologicals against rabies confer almost 100% immunity but once the virus cross the blood brain barrier, death is inevitable. Unfortunately rabies is very common in Pakistan. We don't know the true situation of rabies in the country as no surveillance system exists and it is not reportable disease in Pakistan. The annual estimated number of human deaths in Pakistan ranges from 2000 to 5000 and nobody knows how many animals die due to this horrific disease. To assess the rabies situation and study the circulation of rabies virus in Pakistan, we collected 25 rabies suspected brain samples, amplified and sequenced the rabies virus glycoprotein gene. The sequence analysis revealed that rabies virus movement across the country is very complex phenomenon. Different isolates of arctic strain rabies virus belonging to genotype 1 of rabies virus are prevalent in throughout the country. As preventive vaccination against rabies in domestic animals and wild life does not exist, there is no barrier against the spread of the virus. The rabies isolates move freely across the country, from Pakistan to Afghanistan and India and vice versa. The same isolates of rabies virus with almost 100 % homology are found apparently unrelated locations of Pakistan and Afghanistan so we speculate that Afghan citizen and IDPs who migrate from western border of Pakistan to other parts of country along their animals are involved in the spread of rabies. As 99% cases human rabies occur due to bite of dogs and these dogs acquire rabies virus from these domestic animals and wild carnivore. Therefore we suggest that first step in control of rabies in Pakistan to control the population of street dogs, vaccinates the pet dogs and those migratory animals which move with their owner from one place to other and cause spread the rabies virus in entire country.

Keywords: *rabies; human beings; Pakistan*

ICZ-2014-019

ZOONOTIC DISEASES AN INTERNATIONAL ISSUE: ECONOMIC AND SOCIAL IMPACTS

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History is witness to this fact that Zoonotic diseases had strong bearing on the evolution of man especially those cultures and societies that domesticated and bred animals for food and clothing. Zoonosis had been still the most important risks which mankind is exposed. Zoonotic diseases occur throughout the world transcending natural boundaries. Moreover political changes in the world order and ongoing general agreement on tariffs and trade (GATT) negotiations promise to open new markets for variety of animal products. So Zoonosis no longer is solely a national problem. It is now clearer that with the recognition of inter-relationships between countries, the internationalization of control efforts are being more relevant to technical, economic & social field, especially, when the countries are looking for global competitiveness. Zoonotic diseases do have tremendous social economic impact in respect of considerable losses in production, loss of livelihood of Vulnerable people, including food security. Estimates of global highly pathogenic Avian Influenza loss from the outbreaks since 2003 runs into billion dollars. The cost of outbreak in Hongkong (1997) was \$100s of millions. Preliminary estimates in 2004 of impacts from South-East Asian economies suggested that a single large outbreak could results in reduction of upto 1.5% of GDP growth. In India the impact of 2007 outbreak of Avian Influenza losses in a smaller State of Manipur has been estimated as Rs 1013 lakh on poultry farmers, Rs 1196 lakh on input industry, Rs 34.5 lakh on egg traders and hatchery and Rs 212 lakh on retail outlets. The total worldwide impact of Severe Acute Respiratory Syndrome impact was \$50 billion losses. In U.K, emergence of Bovine Spongiform encephalopathy resulted the Government exchequer net cost of about £3.7 billion. Emergence of Nipah virus in Malaysia Government paid U.S. \$35 million in compensation to the owners and U.S. \$136 billion for control. Approximately 618 homes and 111 shops as well as school and banks were evacuated. In India, besides trade barrier there is estimated loss of Rs350 million due to Brucellosis and more than Rs13000 crores due to Tuberculosis. WHO estimated in 1994 that Plague outbreak in India costing about \$2 billion. Annual loss due to Hydatidosis is estimated to the cattle and buffalo industry of India, costs approximately \$212.35 million. The industry which is one of the largest exporter of meat from India .More impact of emerging Zoonotic diseases of public health significance on economics will be discussed with the aim to have “One Health” paradigm.

Keywords: *zoonoses; international issue; economic impact*

ICZ-2014-020
STRATEGIES TO CONTROL BRUCELLOSIS IN PUNJAB, INDIA

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Punjab is the most dynamic State of India for rearing cattle and buffalo with profitability. The average yield of exotic cows, Indigenous cows as well as Buffaloes in Punjab is highest in the Country. Punjab holds more than 50 lakhs buffalos and about 24 lakhs cattle. The State has extensive cross breeding program targeting exotic breed Holstein Friesian. The State has about 73% of breedable cow population. With the advent of cross breeding program much emphasis is given to control brucellosis. In India, PD_ADMAS (Project Directorate on Animal Disease Monitoring and Surveillance) (2005-2010) reported presence of the disease throughout the country with varying prevalence in different animal species, viz: bovine (22.15%), sheep (8.85%), goat (6.23%), pig (15.35%), yak (16.0%) and mithun (19.0%). This high prevalence of animal brucellosis is responsible for human infections due to close contact with animals. The disease appears to be on increase in recent times, perhaps due to increased trade and rapid movement of livestock and increase in more susceptible high producing cross bred cattle population. The State has embarked the strategies on the control of Brucellosis. State Sero Surveillance Program has been launched in about 19 districts of Punjab State from the year 2011 to 2014. The data indicated on the Sero samples analyzed and ranging from 40,000 to 80,000 that prevalence rate of Brucellosis is less than 6%. Vaccination program has been launched in various parts of the State.

Keywords: *brucellosis; control strategies; Punjab-India*

ICZ-2014-021

RODENTS: THE FOOD CONTENDERS AND POTENTIAL SOURCE OF ANIMAL AND PUBLIC HEALTH DISEASES

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Rodents (Order: Rodentia) are smallest mammals sharing 43% of the global mammal population. They are frequently used as experimental models in research, product testing and studying physiological mechanisms. They are also used for land mines detection. The common species of rodents includes: mice, rats, squirrels, porcupines, beavers, guinea pigs and hamsters. Rodents are synanthrops especially the species namely: house mouse (*Mus musculus*), roof rat (*Rattus rattus*) and Norway rat (*Rattus norvegicus*). Therefore, these can be common reservoirs of zoonotic as well as livestock and poultry infections. Globally, mutilation of crops has been assessed causing nearly 280 million malnourished heads. An expansion in the rodent population in an area can be directly related to the increased zoonotic diseases in human populations. Rodents, as a pest are known to be reservoirs of ticks and tick-borne zoonotic infections of bacterial, viral, and protozoan origin like Lyme disease, babesiosis, rickettsiosis, tularaemia and ehrlichiosis. Rodents have also been connected to the dispersal of emerging diseases like, severe acute respiratory syndrome (SARS) in Asia. Their role in the distribution of trematodes, cestodes, nematodes and protozoa has been well-proven. The rising public health concerns about rodent-borne zoonoses coupled with increasing deterioration of the waste disposal system in many urban and peri urban areas of the world validates an extended research on this important issue. In Pakistan, very scanty data is available on the rodent-borne diseases. Therefore, there is a need to screen rodent population for the prevalence of pathogenic microorganisms through conventional and modern techniques. This will be helpful to devise sustainable integrated disease control programs in the area. The aim of this presentation is to enlighten the significance of rodents in the transmission cycle of different zoonotic infections.

Keywords: *rodents; significance; reservoir; zoonotic diseases*

ICZ-2014-022

TWO NOVEL SINGLE NUCLEOTIDE POLYMORPHISMS IN JAK2 GENE ARE ASSOCIATED WITH LOWER MASTITIS SUSCEPTIBILITY IN DAIRY CATTLE

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Mastitis is the most common and costly disease of lactating animals affecting animal health and welfare, causing economic losses due to treatment costs, lost quarters, loss of animals and poor quality milk. Mastitis is important due to its public health concerns and zoonosis associated risks. Somatic cell count (SCC) is a useful indicator of udder health and has highly positive genetic correlation ($r_g=0.71-0.97$) with clinical mastitis. Numerous therapeutic, prophylactic and management strategies have been proposed as control measures for bovine mastitis; however, a widely proposed strategy is based on improving the host genetics through marker assisted selection. JAK2 gene is located on bovine chromosome 8 and plays crucial role in a variety of inflammatory conditions. The genetic effects of single nucleotide polymorphisms (SNPs) in JAK2 gene were investigated on some serum cytokines and mastitis indicator traits in a population of 268 Chinese Holstein cattle. Pooled DNA sequencing revealed 2 novel SNPs in 3' flanking region of JAK2. Fixed effect model considering the effects of SNPs, parity, herd, season and year of calving was used by general linear model procedure of SAS 9.1. Genotypic frequencies of these SNPs in the population were in Hardy Weinberg Equilibrium ($P>0.05$). The results of association study showed that SNP1 was significantly associated with IFN- γ and SCC, whereas SNP2 was significantly associated with IFN- γ , SCC and SCS ($P<0.05$). Both the 2 SNPs were in LD and 2 haplotypes were revealed between these SNPs. The additive effect of these SNPs was significant on the corresponding traits on which SNP effect was significant. As for mRNA expression analysis, the genotypes of the 2 SNPs significantly associated with higher values of mastitis indicator and serum cytokines traits showed significantly higher mRNA expression than the other genotypes ($P<0.05$). The results imply that the identified polymorphisms could be potentially strong genetic markers to select dairy cattle for genetic resistance against mastitis. Thus improving the host genetics eventually can minimize zoonosis risks associated with mastitis

Keywords: zoonosis; JAK2 gene; SNPs; mRNA expression; mastitis resistance

ICZ-2014-023

SERO- PREVALENCE OF BRUCELLOSIS AMONG THE INDIGENOUS BREEDS OF BUFFALOES IN URBAN, PERI URBAN AND RANDOMLY SELECTED VILLAGES OF DISTRICT PAKPATTAN, PAKISTAN

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Brucellosis has high zoonotic threat for public health as it is one of those zoonotic diseases which are common worldwide. Brucellosis has high impact on economic losses both in terms of animal production and human health. The persons having direct or indirect contact with the infected animals, their products or by-products can acquire infection, because infected animals excrete the pathogenic organism in milk. Keeping in view the zoonotic importance of brucellosis, the present study was designed to investigate the sero-prevalence of brucellosis among the indigenous breeds of dairy buffaloes in populated in urban, peri urban and randomly selected villages of district Pakpattan. The study on sero-prevalence of brucellosis among the indigenous dairy breeds of buffaloes was investigated in urban, peri urban and randomly selected villages of district Pakpattan. A total of 1960 samples (980 of serum and 980 of milk samples) were collected from the urban, peri urban and randomly selected villages of district pakpattan, all the serum and milk samples were screened for the presence of brucella antibodies. The Rose Bengal Precipitation test antigen (RBPT antigen) and Milk Ring Test antigen (MRT antigen) locally manufactured by the Veterinary Research Institute, Lahore, Pakistan were used for the screening of serum and milk samples, respectively. All the serum and milk samples were screened in the district disease diagnostic laboratory Pakpattan, livestock and dairy development department, Punjab, Lahore. The prevalence of brucellosis among the indigenous breed of dairy buffaloes in urban area was investigated as 0.8% (1 out of 125) and in peri urban areas was recorded as 3.23% (11 out of 340). The prevalence of brucellosis in the rural areas was much higher than the prevalence in the urban and peri urban areas of the district Pakpattan 9.7% (50 out of 515). Among the randomly selected villages, the prevalence of brucellosis in buffaloes was much higher (19.9%) in Hotta village of district Pakpattan as inbreeding was very common in that village.

Keywords: *seroprevalence; brucellosis; Pakpattan; Pakistan*

ICZ-2014-024

FISH-BORNE ZOOTIC PARASITIC INCIDENCES IN COMMERCIALY IMPORTANT LANDED FRESHWATER FISH SPECIES OF PAKISTAN

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Fish-borne zoonotic parasites not only pose threat to food security and human health but also may cause significant economic losses in fisheries and aquaculture industry in Pakistan, resultantly the reduced consumer preferences because of food safety disquiet and also restrictions the exports. Fish serve as second intermediate host of food borne trematode infections and serve as direct source of infection in humans. The study was designed to investigate the food-borne zoonotic parasites in commercially food fishes which serve as intermediate host for infection and prevalence of parasites inhabiting in the stomach and intestine of different fish fauna from Sidhnai head works which coordinates 30°34'60" N and 72°4'0" E on Ravi River before its confluence into Chenab River about 15 KM ahead. One hundred and fifty freshly killed specimens of all species were collected on monthly basis for detailed parasitic investigation in the laboratory. Species observed were *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala*, *Hypophthalmichthys molitrix*, *Ctenophryngodon idella*, *Cyprinus carpio*, *Tilapia*, *Wallago attu*, *Notopterus notopterus* and *Aorichthys aor*. Statistical analysis of prevalence showed spatial variation in prevalence of ectoparasites on the fins, skins and gills while the endoparasites were found in the stomach and intestine. Prevalence of nematodes (60%) and trematodes (30%) were higher as compared to the cestodes (10%). 60% incidence for nematodes, 20% for *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala*, and *Hypophthalmichthys molitrix*, respectively while 18%, 8%, 3%, 7% and 4 % for *Ctenophryngodon idella*, *Cyprinus carpio*, *Tilapia*, *Wallago attu*, and *Aorichthys aor*, respectively. *Learnea* was found on the fins, skin and gills in *Catla catla* and *Hypophthalmichthys molitrix*. *Clonorchis* spp. identified in the intestine of *Labeo rohita* and *Cirrhinus mrigala* while protozoan cysts were found in the intestine of *Aorichthys aor*. Parasites were more prevalent in the fish of 8 to 14 cm total length size range. In conclusion, most significant zoonotic parasites were nematodes (*acanthocephalan*, *Sachalinorhynchus* spp), trematodes (*Allocreadium mahaseri*) and cestodes (*Bothriocephalus teleostei*) are identified in fish intestine and stomach

Keywords: *fish-borne zoonotic parasites; commercially important landed fish species; Pakistan*

ICZ-2014-025

A CASE REPORT OF CAPRINE COENUROSI FROM JHANG, PAKISTAN

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This report describes an unusual case of caprine coenurosis in Jhang, Pakistan. A male, crossbred, 8-year old goat, exhibiting nervous signs with subsequent death, represented the typical gross lesions of coenurosis on postmortem examination. The cysts were collected from both cranial and extra-cranial tissues and cautiously examined for definitive diagnosis. Confirmatory diagnosis of caprine coenurosis was validated on the basis of antemortem nervous signs and morphological features of recovered parasitic cysts. To the best of our knowledge, this report provided the first evidence of caprine coenurosis in Jhang, Pakistan. Appropriate deworming of herding dogs could preclude the transmission of coenurosis to small ruminants. Further studies were recommended to determine the prevalence of this parasitic zoonosis on account of potential significance associated with public health and meat quality.

Keywords: *caprine coenurosis; Jhang; Pakistan*

ICZ-2014-026

PREVALENCE OF *TOXOPLASMA GONDII*, *NEOSPORA CANINUM*, AND *SARCOCYSTIS* SPECIES DNA IN THE HEART AND BREAST MUSCLES OF ROCK PIGEONS (*COLUMBIA LIVIA*)

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Little is known about the prevalence of protozoan parasites in the muscles of rock pigeons (*Columbia livia*). The muscles from 54 (heart and breast from 45 and breast only from 9) rock pigeons were examined for DNA of *Toxoplasma gondii*, *Neospora caninum*, and *Sarcocystis* species using PCR. Twenty-four were female and 30 were males. The birds were part of flock of pigeons housed at the tombs of Saints in Lahore, Pakistan. Birds that died or were euthanized due to poor health were submitted for necropsy at the Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, Pakistan where DNA isolations and PCR was conducted. Thirteen (24%) of the birds were positive for *T. gondii* DNA. Seven males and 6 females were positive. Breast tissue was always infected in *T. gondii* positive birds, while the heart was infected in 6 (11.1%) of breast positive finches. Three (5.5%) of the finches, 2 male and 1 female, were positive for *N. caninum*. The distribution of *N. caninum* DNA was more variable in the muscles of house finches than *T. gondii* and was found only in the heart of 1 (female), heart and breast muscle of 1 (male), and only the breast muscle of 1 bird (male). One of the 54 rock pigeons (female) was positive for both *T. gondii* (heart and breast) and *N. caninum* (heart only). *Neospora caninum* amplicons were sequenced and had 9195% nucleotide identity with *N. caninum*. *Sarcocystis* DNA was not found in any bird. The high prevalence of *T. gondii* in rock pigeons and their predation on by cats suggests that they may play an unrecognized role in maintaining environmental contamination and transmission of infection to humans.

Keywords: *Toxoplasma gondii; Neospora caninum; Sarcocystis falcatula; Sarcocystis neurona; Rock pigeons; Columbia livia*

ICZ-2014-027

PREVALENCE OF *GIARDIA INTESTINALIS* AND *HYMENOLESPTIS NANA* IN AFGHAN REFUGEE POPULATION OF DISTRICT MIANWALI, PAKISTAN

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Present study was aimed to investigate prevalence of *Giardia intestinalis* and *Hymenolopsis nana* in Afghan refugees visiting Central Health Unit (C.H.U.), Kot Chandana (Mianwali, Northern Punjab) during two years (February 2007 to December 2009). A total of 687 stool samples were collected from different age groups of both genders. Samples were processed under sterile conditions. Gross examination and microscopic examination was done on same day along with eggs detection after trichrome staining. Significantly higher ($\chi^2=59.54$, $p<0.001$) prevalence of *G. intestinalis* was observed than *H. nana*. Females were found more likely to be infested as compared to males (OR: 1.40, 95% CI=1.03-1.92). Prevalence of both parasites decreased with age and highest prevalence (41.8% and 48.7% respectively for *H. nana* and *G. intestinalis*) was observed in young individuals belonging to 1-15 years of age group ($p<0.001$). Abdominal distress (OR: 1.13, 95%CI=0.83-1.53), vomiting (OR: 1.13, 95%CI=1.13-1.81) and rectal prolapse (OR: 4.26, 95%CI=1.38-13.16) were the gastro-intestinal clinical symptoms observed in *G. intestinalis*. Whereas, bloody diarrhea (OR: 1.56, 95%CI=1.00-2.43) and rectal prolapse (OR: 5.79, 95%CI=1.87-17.91) were found associated with *H. nana* infections. Intestinal parasitic infection is common among Afghan refugees of (Mianwali) Northern Punjab. Prompt preventive measures should be taken for the eradication of high infestation rate which should include clean water supply, public health education, sanitation facilities, promoting personal hygiene and especially periodic deworming of the children.

Keywords: *Giardia intestinalis*; *Hymenolopsis nana*; prevalence; Afghan refugees; Punjab

ICZ-2014-028

Prevalence of Hydatidosis in slaughtered sheep and goats in D.G. Khan Abattoir, Punjab, Pakistan

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This study was conducted from March 2013 to February 2014 to investigate the prevalence of cystic echinococcosis (CE) of slaughtered sheep and goats in D.G. Khan Abattoir, of southern Punjab, Pakistan. The result of this study revealed that a total of 1328 small ruminants (548 sheep and 843 goats) were randomly sampled and examined after slaughter for the presence of CE (hydatidosis) in the visceral organs (lungs, livers and hearts) using the standard meat inspection procedures, where 86 (6.47%) were positive. The positive samples were taken to the laboratory for the cyst identification; fertility and viability test were performed. The study indicated that the prevalence of the CE in the study area was 35 (7.21) in sheep and 51(6.05%) in goats which showed no significant variation between the two species. The distribution of cysts in the internal organs showed significant variation between two organs (Lung and liver) in both animal species $P<0.05$. From the total examined sheep, 24 (68.60%) of the lung, 10 (28.57%) of liver and 1 (2.85%) of the heart which in goats was, 32 (62.74%) for lung, 17 (33.33%) liver, 2 (3.92%) heart, respectively. Lung was the most commonly affected organ both in sheep and goats.

Keywords: *hydatidosis; sheep; goats; lungs; liver; heart; prevalence*

ICZ-2014-029

HUMAN TOXOPLASMOSIS: TRANSMISSION DYNAMICS

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Human Toxoplasmosis is one of the widespread of all the zoonoses. It is caused by the most successful obligate intracellular protozoan parasite *Toxoplasma gondii*. The disease can result in encephalitis, abortion, fetal abnormalities or even prenatal death in immunocompromised individuals. Human acquire infection by consumption of food, raw or uncooked meat or water contaminated with infected cat feces. Vertical transmission from mother to fetus can also occur. Therefore, there is a need to identify the transmission patten and to develop strategies for its control and prevention. The aim of this study has been the understanding of underlying mechanisms of transmission of *Toxoplasma* from definitive host (cats) to women. And, to devise strategies for intervention to break the transmission mode/route at the environment/cellular/molecular level. Initially a total of about 300 blood samples from rural married women belonging to different districts of KP were collected and were tested for the presence of anti-*Toxoplasma* antibodies using ELISA. Information about their life style, occupation, social status, food preference, personal hygiene, contact with cats, association with live stock etc were also recorded. Out of 300 samples 105 (35%) were seropositive for *Toxoplasma gondii*. Among the 105 seropositive women 73 (69.5%) had one or more abortions. All 35% women were in close contact with goats and sheep, consuming unpasteurized milk and contaminated water, and with very poor personal hygiene practices. The study signals alarming situation for married women living in rural set up. The analysis shows that close contact with sheep and goats and consuming unpasteurized milk are more severe risk for human toxoplasmosis than contact with cats as commonly considered a source for spread of disease. Toxoplasmosis is of special significance and requires a thorough investigation at molecular level to understand the genetic diversity of this parasite and the molecular basis of transmission and infection.

Keywords: *toxoplasmosis; human beings; transmission dynamics*

ICZ-2014-030

PREVALENCE AND TRANSMISSION DYNAMICS OF *E. COLI* 0157:H7 IN FARM WORKERS

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Escherichia coli (0157:H7) is a serious threat to human health. Its infection results in outbreaks of haemorrhagic colitis and haemolytic uraemic syndrome. Live stock is the main reservoir. The infection is zoonotic and is transmitted to human through meat and dairy products. The purpose of this investigation was to study the possible risk factors and its transmission pattern. For this, farm workers were tested for the presence of bacteria and to observe their hygiene practice in order to recommend ways to avoid transmission of pathogen to their families and community. The data was collected about their occupation, social status and hygiene practice on a standard CDC questionnaire. The samples from 200 persons at risk were collected from Stool and other exposed body parts. Prevalence calculated was 46% (23) in farm workers, 42% (21) in abattoir workers, 40% (20) in poultry workers and 38% (19) in visitors of these areas. The overall prevalence was 41.5 per hundred people. The isolates of *E.coli* 0157:H7 were tested against five different antibiotics. The bacteria showed resistance to Vancomycin, Gentamicin, and Cefoxitin and were sensitive to Ciprofloxacin and Rifampin. The study confirmed that the people working at animal farms as well as regular visitors are at high risk. Molecular characterization of the isolates is being planned.

Keywords: prevalence; *Escherichia coli*; farm workers

ICZ-2014-031

**PREVALENCE OF FASCIOLOSIS IN BUFFALOES OF BAHAWALPUR,
PUNJAB, PAKISTAN**

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Fasciolosis is a trematode borne parasitic disease that infects liver of large ruminants widely prevalent throughout the world. During the present study fecal samples from buffaloes were collected on random basis from the all tehsils of Bahawalpur district from February 2012 to October 2012. Of total 1800 fecal samples, 284 (15.8%) were found to be positive. Highest prevalence was recorded in Yazman (21.7%) followed by Bahawalpur (16.7%), Khairpur (15.6%), Hasilpur (14.4%) and the lowest was recorded in Ahmadpur (10.6%). Statically chi-square (χ^2) showed non significant ($p>0.05$) difference between all areas. Monthly overall highest prevalence was recorded in September (31%), while the lowest was found in the month of May (3.5%). Statistically a significant ($p<0.05$) difference was recorded in all months. Overall highest seasonal wise prevalence was found in autumn (28.3%) followed by winter (21%), summer (12%) and lowest in spring (8.3%). In age wise prevalence the adult buffaloes were highly (19.9%) infected than young ones (5.3%). Statistically a significant difference ($p<0.05$) was found between all seasons and age groups. Gender wise the prevalence was slightly higher but statistically non significant ($p>0.05$) in females (15.9%) than males (15.1%). Bahawalpur (Pakistan) has a significant prevalence (%) of fasciolosis that may cause economic loss.

Keywords: *fasciolosis; buffaloes; Bahawalpur; prevalence*

ICZ-2014-032

PREVALENCE OF *MYCOBACTERIUM BOVIS* IN HUMAN POPULATION IN RAWALPINDI, PAKISTAN

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The causative agent of bovine tuberculosis, *Mycobacterium bovis* is also reported to cause disease in human beings. With the advent of immune suppressing diseases like HIV/AIDS, new questions have been raised regarding epidemiological impact of the transmission of tuberculosis in and between human beings. Although eradicated on vast level in developed countries, the disease widely occurs in humans in developing countries and is often neglected. Because of animal and public health concerns, proper surveillance programs should be carried out on prior basis especially in high risk factor areas. Current study aimed at identifying certain factors affecting human tuberculosis and estimation regarding disease prevalence in district Rawalpindi, Pakistan. The study was conducted for a total duration of six months. Interviews and medical examinations of individuals were carried out. Assessment was done regarding association of age, sex, poverty, birth place and herd keeping with the disease. Incidence of tuberculosis was high in adults than children and young ones. Cases notified showed that the disease is less frequent in females. Poor people and those who were in contact with large animals were at higher risk of tuberculosis.

Keywords: *Mycobacterium bovis; human beings; Rawalpindi-Pakistan*

ICZ-2014-033

EXPERIMENTAL INFECTION OF HEPATITIS E VIRUS IN MICE

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Hepatitis E virus (HEV), a zoonotic pathogen cause acute hepatitis, several animal species can reportedly act as reservoirs. HEV and antibody to the virus have been detected in a variety of animals. HEV isolated from infected pig livers brought from Hebei, China. Male Gerbil mice were inoculated with swine HEV isolated from infected liver, via intraperitoneally and intravenous injection to insure infection. Negative control groups of mice were also included in the study. The liver, spleen, kidney, jejunum, ileum, cecum, colon and testicles of each mouse from all three groups were collected for reverse transcription nested polymerase chain reaction (RT-nPCR) detection, and histopathologic examination. The sera from mice were tested for anti-HEV IgG by enzyme linked immunosorbent assay (ELISA). Activities of liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST) were also measured in the sera of the mice. HEV RNA was detected in liver, spleen, kidney, jejunum, ileum and colon and testicles by RT-nPCR in all of the inoculated groups. Tissue antigen was observed immunohistochemically in the different cells of various organs. Histopathological changes were observed in the liver, spleen, kidneys and testicles of these mice showed marked obvious changes. Infected mice showed increased levels of AST, and anti-HEV IgG in sera. The result showed that Gerbil mice could be readily infected by HEV isolated from pigs that can be zoonotic also.

Keywords: *Hepatitis E; mice; experimental infection*

ICZ-2014-034

IMPACT OF BIRD FLU EPIDEMIC ON CONSUMPTION PATTERN OF CHICKEN MEAT IN DISTRICT LAHORE, PAKISTAN

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This paper seeks to identify the major factors affecting consumer demand of chicken meat in context of bird flu epidemic. Data was collected by personal interview method from the consumers of chicken meat, selected randomly from the Lahore city. The impact of independent variables (education, age, family size, income and low income consumers) was estimated using logit regression analysis. The highest odd ratio (1.60) was found for the dummy variable used to capture effect of low income on consumer demand for chicken meat followed by family size (1.45), education (0.98), income (0.96) and age (0.91). The study emphasizes the role of public and private regulatory authorities to take measures for controlling bird flu and rumors related to it which shake confidence of consumers in purchasing chicken meat.

Keywords: *bird flu; chicken meat; poultry; demand; logistic regression*

ICZ-2014-035

RECURRENT OUTBREAK OF CUTANEOUS LEISHMANIASIS AT MULTAN, PAKISTAN

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After the attack of KPK Karak, Khot area, in January and February 2014, by sand fly causing Cutaneous leishmaniasis effecting more than 300 patients it droned on Multan. In and around, the habitants of Multan local area surround the Multan Gates like Lohari Gate, Dolat Gate, Dehli Gate and the suburbs more than 14 patients suffering from attacks of Sand fly leading to Vector born disease Cutaneous Leishmaniasis in just 30 days starting from 13 February, 2014. This could leads to epidemic if unleashed and an alarming situation could cause insomnia to residents of Multan. The adjoining area and new Towns could be attacked by the pest due to the ideal filthy situation of the municipality. The study has been planned to adopt control measures and to curtail the outbreak that is re-occurring due to the presence of vector and zoonotic protozoa.

Keywords: *outbreak; leishmaniasis; Multan; Pakistan*

ICZ-2014-036

SERO-PREVALENCE STUDY OF BRUCELLOSIS CAUSED ABORTION IN CAMELS IN SANDY AREAS OF SOUTHERN PUNJAB, PAKISTAN

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Brucellosis is a very infectious and zoonotic disease. A massive population of female camels is aborted due to brucellosis in any trimester especially the last trimester. Whole investment on animal at the end leads to heavy losses not only to new camel generation but also a great economic failure to the camel owner. Not only female camels but also male camels carry the disease. Regular screening and identification may prevent the infection among the camel herd as well as decrease the risk in human beings. This disease is one of major cause of infertility in the animals especially the camel. Human infertility risk due to its zoonotic effect in camel farmer, veterinarian and AI technicians can only be controlled by regular test and screening program to control havocs of this disease. It causes damaging effect on the reproductive health of human being especially low income camel breeders. As treatment and prevention strategy of the disease in the camels and camel farmers is the worst in deeply populated areas of camels like Cholistan and Thar. There is a colossal population of camels and camel breeder which may be affected and remain unnoticed. It needs the proper attention from the state to establish the proper surveillance, eradication and prevention programs for brucellosis.

Keywords: *seroprevalence; brucellosis; abortion; camel; zoonosis; Punjab*

ICZ-2014-037

EMERGING ZONOTIC THREAT: HIGH NUMBERS OF MULTIDRUG RESISTANCE EXTENDED SPECTRUM BETA LACTAMASES (ESBLs) *E. COLI* IN MIGRATORY BIRDS IN PAKISTAN

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Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan

ESBLs producing bacteria have become major concern in humans and veterinary medicine due to their resistance to third generation cephalosporins. Migratory birds could be the reservoirs and potential source of spread of ESBLs producing *E. coli*. In the present study, we focused on the prevalence of ESBLs producing *E. coli* in migratory avian species, which arrives in Pakistan from Siberia and Central Russia via international migratory bird-route number 4 or Indus flyway. A total of 100 migratory birds fecal swabs were collected during October 2013-Feb, 2014 in Pakistan. These birds mainly include mallard (n=37), Eurasian coot (n=17), starlings (n=14), common pochard (n=9), shovler duck (n=6), gadwell ducks (n=5), quail (n=5), red-headed pochard (n=4) and wigeon duck (n=3). The samples were screened for ESBL by cultivation on CHROMagar-ESBL (CHROMagar, France). All isolates were phenotypically confirmed as ESBL using antibiotic sensitivity testing and double disk synergy tests according to CLSI criteria. All isolates were biochemically confirmed as *E. coli* using commercial Remel RAPID ONE test (Remel, UK) and genotypically by *uid* gene detection with PCR. Presence of *bla*_{CTX-M}, *bla*_{TEM}, and *bla*_{SHV} genes was confirmed using PCR. In addition, resistance to gentamycin, chlorempenicol, tetracyclin, sulfmethoxazole and nalidixic acid were also tested. A total of 40 out of 100 (40%) migratory birds carried ESBL *E. coli*. All isolates were positive with *uid* gene for *E. coli*. Highest number of ESBL producing *E. coli* were found in mallard n=15 (37.5%), Eurasian coot n=7 (17.5%), common pochard n=6 (15%), starling n=3 (7.5%), Shovler duck n=2 (5%), gadwell duck n=2 (5%), red head pochard n=2 (5%), wigeon duck n=2 (5%) and quail n=1 (2.5%). PCR showed *bla*_{TEM} as the most frequent ESBL type 50% (20/44) followed by *bla*_{CTX-M} 23% (9/40). Out of 9 *bla*_{CTX-M}, 8 also carried *bla*_{TEM} gene whereas *bla*_{SHV} was found only in one ESBL *E. coli*. Among the other drugs tested, nalidixic acid, tetracycline and sulfmethoxazole showed highest resistance. This is the first report in Pakistan showing migratory birds as reservoirs of ESBL *E. coli*. The significantly high rates of ESBL *E. coli* in migratory birds could be a potential source of zoonosis and transboundary spread of ESBL *E. coli*. Further studies are needed to unravel phylogenetically important clone types among ESBL *E. coli*.

Keywords: ESBL *E. coli*; multidrug resistance; migratory birds; Pakistan

ICZ-2014-038

ISOLATION AND IDENTIFICATION OF *BRUCELLA* FROM ABORTING EWES

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Brucellosis is an important bacterial disease affecting productivity of domestic animals through abortion, metritis, retention of placenta, infertility, orchitis and epididmitis in many developing countries including Pakistan. It is also considered as important zoonoses. A suspected brucellosis outbreak was investigated at a sheep farm near Taxila tehsil Rawalpindi when a local veterinarian reported frequent abortions in sheep flock. The flock composition was 240 ewes and 10 rams. The clinical examination of flock and sampling was conducted. The 141 ewes and three rams were bled for sera collection by jugular vein puncture. In addition abomasal contents from three aborted foeti were also collected. The sera samples were examined for the presence of *Brucella* specific antibodies using competitive ELISA. Of 144 samples tested 23 (22.1%) were found positive for brucellosis. Milk samples (10) were collected from lactating ewes which were found positive by cELISA. The milk samples and abomasal contents of the aborted foeti were inoculated onto trypticase soya agar for isolation of *Brucella melitensis*. Suspected *B. melitensis* colonies which appeared after 7 days of incubation, were smooth, glistening, bluish and translucent. The *B. melitensis* colonies were identified using staining reaction (Gram's and modified Ziehl-Neelsen staining), biochemical characteristics and PCR. Under microscope organisms appeared as red coco-bacilli with both staining techniques. The isolates were oxidase and catalase positive. Two *B. melitensis* isolates were recovered from milk samples of two different animals. It was concluded that *B. melitensis* is prevalent in sheep flocks as an important cause of abortion. Furthermore, excretion of *B. melitensis* in the milk of infected ewes may represent an important source of transmission to other animals and humans.

Keywords: *Brucella; ewes; isolation; identification*

ICZ-2014-039

ELISA BASED DETECTION OF *ENTAMOEBIA HISTOLYTICA* FROM SEWAGE AND TAP WATER SAMPLES IN AN ENDEMIC AREA OF LAHORE, PAKISTAN

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Entamoeba (E.) histolytica, a ubiquitous protozoan parasite, is the causative agent of amoebiasis in humans. It is a widespread waterborne pathogen in areas where barrier between human feces, soil, food and water source are ordinary. In current study 638 sewage and tap water samples from Lahore, Pakistan were collected and processed to detect amoebic cysts using the light microscopy and antigens of *E. histolytica* were detected using a antigen ELISA. Because it is impossible to differentiate *E. histolytica* from *E. dispar* using light microscopy we referred to all cysts morphologically consistent with *E. histolytica* as *E. histolytica*-like to reflect this uncertainty. 284 samples from sewage water and 354 tap water samples were examined. *E. histolytica*-like cysts were observed in 108 (16.9%, 95% CI= ±3.98) by triple fecal test microscopy and *E. histolytica* antigens were demonstrated in 86 (13.47%, 95% CI= ±4.63) by antigen ELISA in 638 samples from sewage and tap water. Seventy-one (25%) and 53 (18.6%) of 284 samples from sewage water were positive by microscopy and by antigen ELISA, respectively. Thirty-seven (10.54%) and 29 (9.32%) of 354 samples from tap water were positive by triple test and by antigen ELISA, respectively. Significant difference ($P \leq 0.05$) in prevalence was observed between the samples from sewage and tap water. Prevalence was significantly higher ($P \leq 0.05$) in summer season than other three seasons. These findings suggest that presence of this pathogen in sewage and tap water may play an important role in the epidemiology and transmission of this organism.

Keywords: *Entamoeba histolytica*; protozoan; pathogen; cyst

ICZ-2014-039

ELISA BASED DETECTION OF *ENTAMOEBIA HISTOLYTICA* FROM SEWAGE AND TAP WATER SAMPLES IN AN ENDEMIC AREA OF LAHORE, PAKISTAN

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Keywords: *Entamoeba histolytica*; protozoan; pathogen; cyst

ICZ-2014-041

PRIONS: AN EMERGING ZONOSIS

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Prions are the infectious proteins which are transmitted among hosts by three different ways acquired, familial or sporadic. The normal prion protein (PrP) of the cell becomes infectious (PrP^{sc}) through a post-translational change in which a β -sheet is added in their structure. The disease is mainly a food borne dilemma and known to transmit via ingestion of infected animal meat. It is a creeping neurodegenerative disease which is recognized by behavioral changes and its incubation period varies from months to year. On postmortem, there are no apparent changes in the brain but microscopically there is spongiform degeneration and astrocytic gliosis. In humans, it is found in four different forms, Creutzfeldt-Jakob Disease (CJD), Gerstmann-Straussler-Scheinker Syndrome (GSS), Fatal Familial Insomnia, Kuru. CJD has further classified into Classical Creutzfeldt-Jakob Disease (cCJD) and Variant Creutzfeldt-Jakob Disease (vCJD). Regardless of many efforts to find a treatment, the diseases caused by prions are incurable, yet trials are continue and soon scientists will find a solution to control this malady.

Keywords: *Creutzfeldt-Jakob disease; prions; zoonosis*

ICZ-2014-042

**HELMINTHOZONOSIS BY *TOXOCARA* SPECIES: ITS CLINICAL FEATURES,
DIAGNOSIS, THERAPEUTIC APPROACH AND PREVENTIVE MEASURES**

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Toxocariasis is an ignored but still a common zoonotic clinical infection of human beings caused by *Toxocara canis* or *Toxocara cati* species of dogs and cats respectively. First human case was noticed in 1950 as an undefined species of nematode from retinal granuloma of a baby. Over all sero-prevalence is higher in tropic regions up to 92.8% as compared to temperate regions i.e. up to 37%. Eggs of *Toxocara* can survive in the environment for long due to thick walled shell and humans can be infected through accidental ingestion from contaminated soil, vegetables or water. Clinically, it produces visceral and ocular larval migrans and granulomatous lesions on viscera and various parts of eye in children of 5-10 years age. In adults it produces generalized symptoms of hypersensitivity and mostly remains undiagnosed. Most common diagnostic technique is enzyme linked immunosorbant assay (ELISA) by using specific toxocara antigen proteins and it can be treated through deworming and surgical removal of larvae. Moreover, it was seen that unhealthy pets are the main contributing sources of contamination so regular deworming of pets and routine personal hygienic measures should be adopted to render the world as *Toxocara* free infection.

Keywords: *Toxocara spp.; helminthozoonosis; therapy; prevention*

ICZ-2014-043

SEROPREVALENCE OF *TOXOPLASMA GONDII* IN SLAUGHTER HOUSE WORKERS AND CATTLE IN LAHORE, PAKISTAN

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Present study was conducted to check the seroprevalence of *Toxoplasma gondii* infection in slaughterhouse workers and transmission from animals (cattle) to humans in Lahore, Pakistan. A total of one hundred blood samples were collected (50 samples from slaughterhouse workers and 50 from cattle) from local slaughter houses and local farms dairy farms around Lahore. Seroprevalence by antibodies detection of *Toxoplasma gondii* was assessed by using Latex agglutination test (LAT). In present study, an overall 10.0% of slaughterhouse workers and 14.0% animals (cattle) were found seropositive for *Toxoplasma gondii* infection. Slaughterhouse workers in the age group of 51-60 years & above (n=12) were exhibited highest seropositivity (25.0%) while workers in the age group of 41-50 years (n=10) followed seropositivity (20.0%). Similarly seropositivity in animals (cattle) (14.0%) was also related to age (more than 5 years) by using Latex agglutination test (LAT). Seroprevalence of *Toxoplasma gondii* infection in animals and workers indicated its importance in transmission from animals to humans.

Keywords: *toxoplasmosis; seroprevalence; cattle; zoonoses*

ICZ-2014-044

PREVALANCE OF BOVINE BRUCELLOSIS IN COWS OF HAFIZABAD DISTRICT OF PAKISTAN

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A total of 782 samples of milk and serum were challenged against MRT, RBPT and SAT for the detection of Brucellosis in cows during the span of five years since 01-07-2009 to 30-06-2014. Animals were divided into two groups i.e., Aborted Animals and Repeaters. Each of these was further sub-divided into three groups i.e. MRT Group, RBPT Group and SAT Group. It was found out that the ratio of abortion due to brucellosis in cows is 31.164% while ratio of repeating insemination was 36.363%.

Keywords: *zoonosis; brucellosis; abortion; bovines*

ICZ-2014-045

RECOVERY OF *SCHISTOSOMA HAEMATOBIMUM* OVUM FROM *LABEO ROHITA*: FIRST REPORT FROM PAKISTAN

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Schistosomiasis, also known as bilharzia, is a parasitic disease caused by trematodes from the genus *Schistosoma* that can infect humans and animals. *S. mansoni*, *S. japonicum*, and *S. mekongi* all causes intestinal schistosomiasis except *S. haematobium* that causes urinary schistosomiasis. It is only specie which effects urinary system, it can affect liver, heart, lungs also but very rarely. *Schistosoma haematobium* is endemic to over 50 countries in Africa and the Middle East and Western Asia and may be fatal in HIV positive people. A number of reports from the African countries like Nigeria have been reported. A few cases are reported² but in Pakistan it has never been reported before in native people. It is first time reported in Pakistan in the intestine of Rahu (*Labeo rohita*). The purpose of this study is to elaborate the approach of zoonotic agent by various other routes including the commonly available fish.

Keywords: *Schistosoma; Labeo rohita; first report; Pakistan*

ICZ-2014-046

STUDY ON SERO- PREVALENCE OF BRUCELLOSIS AMONG THE BUFFALOES AND CATTLE IN URBAN, PERI URBAN AND RURAL AREAS OF DISTRICT MANDIBAHUDDIN, PAKISTAN

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Brucellosis is a highly important zoonotic disease and it has great impact on public health. The human beings that have direct or indirect contact with the animals infected with brucellosis may acquire the infection, as the infected animals secrete the causative agent into the milk and vaginal secretions. The present study was designed to determine the sero-prevalence of brucellosis among the indigenous breeds of dairy buffaloes and cattle of district Mandi Bahuddin. The study was conducted from July, 2012 to June, 2013. The sero-prevalance of brucellosis was investigated among the indigenous dairy breeds of buffaloes and cattle, populated in urban, peri-urban and rural areas of district Mandi Bahuddin.

In this study, a total of 1540 serum samples (770 of buffaloes and 770 of cattle serum samples) were collected from the urban, peri-urban and rural areas that were highly populated with livestock populations. The screening of all the serum samples (buffaloes and cows) for the presence of brucella antibodies was done with the Rose Bengal Precipitation test antigen (RBPT antigen), that antigen was purchased from the Veterinary Research Institute, Lahore, Pakistan. All the serum samples of cows and buffaloes were analyzed in the district disease diagnostic laboratory Mandi Bahuddin, livestock and dairy development department, Punjab, Lahore. The prevalence of brucellosis among the indigenous breeds of dairy buffaloes and cows in urban areas was recorded as 01.82 % (2 out of 110) and 0.91% (1 out of 110), respectively. While the prevalence of brucellosis was investigated as 5% (9 out of 180) and 3.89% (7 out of 180), in buffaloes and cows, respectively. The prevalence of brucellosis among both of the buffaloes 7.29% (35 out of 480) and cows 5.83% (28 out of 480) was significantly greater in rural areas than in urban and peri urban areas of Mandi Bahuddin. In the present study the prevalence of brucellosis in buffaloes was higher than that in cows; similarly it was also investigated that the prevalence of brucellosis was also higher in rural areas as compared to urban and peri-urban areas, because natural mating (inbreeding) is very common in rural areas, so the infected bull may be the source of disease spreading.

Keywords: *brucellosis; buffaloes; cattle; seroprevalence; Mandi Bahauddin; Pakistan*

ICZ-2014-047

OCCURRENCE OF *SALMONELLA* IN FRESHWATER FISH WITH ZOONOTIC IMPLICATIONS

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Fish accounts for about 17 percent of the global population's animal protein source intake. Fishery products, however, have been recognized as a major carrier of food-borne pathogens. Human infected by pathogens transmitted from fish is fairly common. Fish serve as a host to a variety of parasites including *Salmonella* spp. that is a worldwide public health issue. *Salmonella*, usually, is not a fish pathogen, rather the consumption of *Salmonella* contaminated feed and water causes this infection. Smoked fish serves as a vehicle for the transmission of *Salmonella* species which exists on skin, gills and intestine. In the absence of application of suitable hygiene for the control of *Salmonella* in the fisheries production sector may be the main reason for the spread of the diseases. The impact of this pathogen in human, particularly in the very young or elder includes gastroenteritis, abdominal cramps, enteric fever, vomiting and bacteremia resulting from ingestion of uncooked fish. In spite of these health impacts, their epidemiology is still poorly understood. Studies are, therefore, needed to summarize the impact of *Salmonella* infected fish in human, with particular attention to freshwater fishes.

Keywords: *human pathogen; Salmonella; contaminated feed; freshwater fish*

ICZ-2014-048

SERO-PREVALENCE AND ZOONOTIC POTENTIAL OF *TOXOPLASMA GONDII* IN CAMELS IN CHOLISTAN DESERT AREA OF BAHAWALPUR, PAKISTAN

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The present study was conducted to find out the sero-prevalence of toxoplasmosis in camels and their owners in cholistan desert area of Bahawalpur, Pakistan. Serum samples from camels (n=226) and humans (n=113 camel owners and n=113 people having no contact with camels) were collected and analyzed by using Latex Agglutination Test (LAT). Overall 9.73% camels were seropositive for anti-toxoplasma antibodies. In the month of April camels had the high prevalence of 11.5% (9 out of 78). The highest prevalence (12%) was detected in camels in the 11 year or above age group. The prevalence of toxoplasmosis was high in the third trimester of pregnancy (27.2%). Overall 27 (12%) humans were seropositive for anti-toxoplasma antibodies. The highest sero-positivity was observed in camel owners (15.92%) and the lowest sero-positivity (7.96%) was observed in the people having no contact with camels. Statistical analysis showed that there was significant difference (P<0.05) between pregnant camel, positive and negative sample groups. Statistical analysis revealed that there was significant difference (P<0.05) in the presence of anti-toxoplasma antibodies between camel owners, positive and negative sample groups. In conclusion, *T. gondii* infection is prevalent in camels of cholistan desert area of Bahawalpur and has zoonotic potential for camels owners in the area.

Keywords: *Toxoplasma gondii; seroprevalence; camels; Cholistan; Pakistan*

ICZ-2014-049

DIVERSITY AND ABUNDANCE OF ZOOPHAGOUS INSECTS IN AND AROUND ISLAMIA UNIVERSITY CAMPUS, BAHAWALPUR, PUNJAB, PAKISTAN

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Zoophagous insects constitute a major part of predatory fauna. Predators either generalist or specialist plays an important role in maintaining the natural balance of an ecosystem. Insects belonging to different orders viz. (Odonata, Coleoptera, Diptera and Hymenoptera) either present on the ground or on leaves were collected from Baghdad-ul-Jadeed campus. The insects were collected from different habitats as (desert area, vegetation, landscapes, crop fields and grassy lawns). Specimens were captured by direct hand picking, with the help of hand nets and automatic sifters. Later they were preserved in solution (Alcohol + few drops of Glycerin). Statically the taxonomic status was determined with the help of available literature in keys and online web sites. These findings seemed to be helpful in ecological management of the ecosystem.

Keywords: *insects; zoophagous; diversity; abundance*

ICZ-2014-050

PREVALENCE OF *CAMPYLOBACTER* IN RAW BOVINE MILK COLLECTED FROM DISTRICT MULTAN, PAKISTAN

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Campylobacter spp. is one of the most frequent pathogens of acute bacterial gastroenteritis, which is transmitted mostly via food originating from animals. It may lead to Guillain-Barre syndrome, which is a serious neurological disease with symptoms that include flaccid paralysis. The present study was conducted to determine the prevalence of *Campylobacter* spp. in raw bovine milk in and around Multan. 120 raw bovine milk samples, randomly selected from dairy bovine herds in Multan, were evaluated for the presence of *Campylobacter*. In this study, 38 of 120 raw bovine milk samples (32%) were found to be contaminated with *Campylobacter*. *Campylobacter* isolates recovered from raw bovine milk were all identified as *C. jejuni* and *C. Coli*. These results suggested the presence of *Campylobacter* spp. in bovine raw milk that can be a serious risk factor for human health. The contaminated milk samples can play an important role in the zoonoses and maintenance of this pathogen in the environment.

Keywords: *Campylobacter; zoonoses; bovine milk; Multan*

ICZ-2014-051

MAGNITUDE OF CAMPYLOBACTER INFECTIONS AND ANTIBIOTIC RESISTANCE IN POULTRY MEAT AND ITS CORRELATION WITH CAMPYLOBACTERIOSIS IN HUMANS

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In the present research investigation, a total of 284 spatially and temporally related *Campylobacter* isolates, including 112 from clinical human cases (*Campylobacter jejuni* 88; *Campylobacter coli* 34) and 172 from retail meats (*C. jejuni* 102; *C. coli* 70), were analyzed for antimicrobial susceptibilities, and virulence. Ciprofloxacin-resistant *C. jejuni* was observed in 23% and 37% of the isolates from humans and retail chicken breasts, respectively. Antimicrobial resistance to ciprofloxacin and erythromycin was detected in *C. coli* isolates recovered from 34% and 21% of retail meats and 18% and 9% humans, respectively. Overall, virulence determinants were more prevalent in *Campylobacter* isolates recovered from retail meats than from humans. In conclusion, significant differences observed in the distribution of antimicrobial resistance profiles, virulence determinants, and genotypic diversity among *C. jejuni* and *C. coli* isolates indicate that there are sources other than retail meats that may also contribute to human Campylobacteriosis.

Keywords: antimicrobial resistance; *Campylobacter*; human; retail meats

ICZ-2014-052

INFLUENCE OF SEASON, AGE AND SEX ON PREVALENCE OF *LEISHMANIA SPP.* IN RATS AND MICE OF LAHORE CITY, PAKISTAN

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A number of zoonotic protozoa are harbored by rodents especially rats and mice. The situation is further aggravated by the higher incidence of rodent borne diseases. Vector proliferation in high human density and low-income urban areas is the consequence of a number of factors such as inadequate housing facilities, unhygienic water supply, poor sanitation and lack of solid waste management facilities as well as the attitude and customs of the people. A total of 3600 rats and mice were examined for the prevalence of various zoonotic parasitic infections. Species of various parasites were identified. The order of infection rate was as *Leishmania spp.* (1.2%). The highest (4.7%) month-wise prevalence of *Leishmania spp.* was found during July and August whereas infection was absent during January, February, March, April, October, November and December. The highest (3.4%) season-wise prevalence was noted during summer followed by autumn (1.1%) then spring (0.1%) and infection was absent during winter. The infection was higher in females (1.4%) than males (0.9%). The infection was found only in adult rats and mice and no positive case was recorded in younger. The infection was (1.2%) in rats and mice. This study will be helpful to disseminate information regarding zoonotic potential of parasitic infections via media i.e. newspapers, magazine, electronic media (T.V, I.T, Radio) through seminars, meetings and conferences with professionals doctors, paramedical staff and livestock farmers.

Keywords: *Leishmania; rodents; season; age; sex; Lahore*

ICZ-2014-053

RODENTS FROM URBAN HABITATS OF LAHORE PAKISTAN AS ZONOTIC VECTORS OF *HYMENOLEPIS NANA*

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Rats/mice act as main reservoir and source of infection in different rural and urban areas of the world. *Hymenolepis nana*, a dwarf tapeworm is a common parasite in small intestine of the rodents and its prevalence is much higher in populations under normal conditions due to its character of autoinfection. Potential of rodents to act as vector in the transmission of *Hymenolepis nana* was assessed by examining the trapped rats (n=3190) and mice (n=410) from three areas (n=3600) of Lahore-Pakistan. Prevalence of *H. nana* detected in both rats and mice was 59.6%. Level of *H. nana* infection was higher in rats (65.1%) in comparison to mice (16.3%). Rodents were differentiated as male (1536) and female (2064) according to their sexual organs. Percent infection with *H. nana* was higher in male rodents (78%) than female (45.9%). Infection level was significantly higher in adult rodents (66.5%) than young (6.8%). The highest (78.0%) month-wise prevalence of *H. nana* was observed in August and lowest (43.7%) in February. This study is helpful to disseminate information regarding zoonotic potential of rodents in transfer of *H. nana* to human population. It is concluded that rodent populations at different localities as well as structures are infected by this zoonotic parasite and human beings are residing in association with them in Pakistan.

Keywords: rodents; *Hymenolepis nana*; zoonotic vector; urban areas of Lahore; Pakistan

ICZ-2014-054

PATHOLOGICAL STUDIES ON CAPRINE BRUCELLOSIS (*BRUCELLA MELITENSIS*) IN DISTRICT SHANGLA, KHYBERPUKHTUNKHWA, PAKISTAN

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In the present study two serological tests namely Rose Bengal Plate Test (RBPT), and Polymerase Chain Reaction (PCR), were used for the detection of the *Brucella melitensis* in goats at District Shangla. During present study 200 blood (serum) samples were screened for brucellosis by RBPT, in which 100 samples were taken from Does and 100 from bucks. The prevalence of brucellosis by RBPT was 13.5 % in both sexes. PCR showed prevalence % lower than the RBPT e.g. 11.5 %. Low degree of prevalence was found in male than the female e.g. 13 cases out of 100 and 11 cases out of 100 respectively. In the same way low degree of prevalence 10 percent was found in goats having age below one year as compared to those who have age more than one year e.g. 13%. The overall Seropositivity by RBPT and PCR was 13.5 percent and 11.5 percent respectively. The diagnostic accuracy of the RBPT was compared with the gold standard test PCR because it can detect more positive cases as compared to RBPT. The sensitivity of RBPT was 62.22 percent while its specificity was 92.66 percent. The positive predictive value and negative predictive value of RBPT were 53.57 percent and 95.35 percent respectively. Furthermore over all eight cases were detected positive by PCR which were declared negative by RBPT. Moreover thirteen RBPT positive cases were declared negative by PCR. The p-value which was 0.63 for RBPT and 0.61 for PCR which was greater than 0.05 indicating that the difference was statistically non-significant. The most frequent gross lesion observed in the study was endometritis along with its different forms. The other lesions included, inflammation of uterine horns, cervicitis (5%), edema (7%), necrotic foci ((5%), yellowish exudates (9%) and ulceration (8%). While in case of male gross lesions were observed in the testicle of only 3 bucks. The testicles were enlarged 2 times than normal and the tunica vaginalis was diffusely adherent to the tunica albuginea. Gross examination of spleen in both sexes showed that

spleen was found to be somewhat enlarged. Its capsule was very tense and it has an intensely dark slaty colour in two cases (13.4%). The tension on the capsule was great that in some very sever and pernicious cases it was observed as thin as muslin and with the slightest pressure of the knife to cut through it led to its rupture. In same way gross examination of inguinal lymph nodes of both sexes revealed that these nodes were relatively large in size and firm in consistency. However four (26%) case appeared edematous and on cut section watery exudates

oozes. The histopathological examination revealed that the ratio of the leucocytes infiltration was higher followed by hemorrhages, necrosis hypertrophy of endometrial glands, degeneration and congestion. The hematological results showed that the values of TLC, TEC, PLT, MCV, PCV, MCH, MCHC, Hb, lymphocytes and monocytes remained unchanged. Thus the result revealed that *Brucella melitensis* is not responsible for any significant change in hematological values. It is concluded that brucellosis is prevalent slaughtering goats at slaughter house and considerable attention is to be needed for proper screening of these goats.

Keywords: *brucellosis; goats; pathology; hematology*

ICZ-2014-055

SEROPREVALENCE OF BRUCELLOSIS AMONG HIGH RISK INDIVIDUALS OF SOUTHERN PUNJAB, PAKISTAN

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The present study was conducted to know the seroprevalence of brucellosis among high-risk individuals consisting for para-veterinary staff and butchers. A total of 436 blood samples from butchers (n = 253) and para-veterinary staff i.e. Veterinary Assistants/ AI Technicians (n = 183) were collected from District of Multan and Bahawalpur during April to June 2014. Blood samples were centrifuged and serum was divided into aliquots and stored at -20°C until tested for presence of Brucella antibodies. Out of 253 butchers, 31 (12.25%) showed presence of antibodies against Brucella antigen by RBPT and SAT method. Similarly, 11 (6.01%) para-veterinary staff showed presence of antibodies. A significantly higher seroprevalence of brucellosis among butchers was found as compared to para-veterinary staff (P < 0.05). In second part of study all blood samples were tested through enzyme-linked immunosorbent assay (ELISA) for the presence of brucella antibodies. The prevalence of brucellosis among butchers and para-veterinary staff was 17% and 11% respectively. It was concluded that ELISA is more sensitive test than the conventional tests and there is an abrupt need to introduce strategies to control brucellosis in Southern Punjab.

Keywords: *brucellosis; seroprevalence; RBPT; SAT; ELISA; zoonosis*

ICZ-2014-056

ORGANIC BROILER PRODUCTION, AN OBJECTIVE TO DIMINISH FOOD BORNE ZOOSESES

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The commercial poultry sector of Pakistan contributes about 987000 tons of meat, 14556 millions of eggs and Rs. 200 billion annually. The contribution of this sector is 1.3 percent in National GDP showing its value addition as 6.1 percent in Agriculture and 10.8 percent in Livestock sector. Salmonellosis, Campylobacteriosis and Bird flu (Avian Influenza) are among the most important food borne diseases from commercial poultry causing serious health issues in humans. Organically produced broiler meat and layer eggs (produced in natural environment; free from any type of synthetic chemicals, biological, growth promoters) can decline the loads of pathogenic/ zoonotic organisms. In spite of commercial/ conventional poultry farming, organic broiler production needs high attention with respect to bio security and addition of any biological from animal origin which can decrease the chances of food borne Zoonoses. Still this sector requires passionate attention to explore the true desires for commercial organic broiler production to minimize the health risks and feasibility of production as well.

Keywords: food borne zoonoses; Salmonellosis; Campylobacteriosis; bird flu; organic broiler production

ICZ-2014-057

PREVALENCE OF INTESTINAL PARASITES AND RISK PERCEPTION OF ZONOTIC INFECTION FOR HUMANS

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The study consisted of two parts on various related aspects from Karachi during, 2013. The results for the first part of study indicated that the overall prevalence of gastrointestinal parasites was 86.00%, the GI parasitic species identified were hook worm, tape worm, round worm and *Giardia protozoa*. The results of the second part of the study included the survey, in which 15 veterinary practitioners were asked about the significant parasite in their practice area. Out of 15 veterinarian surveyed, the *Toxocara canis* was considered as a significant problem by 01 veterinarian; Hook worm (*A. caninum*) by 5 veterinarians, *Echinococcus granulosus*, *Dipylidium caninum* and *Trichuris vulpis* by 5 veterinarians each, *Taenia / spirometra* by 3 and *Giardia* spp. by 09 veterinarians, who mentioned these as significant problems. In case of zoonotic awareness of dog owners, the zoonoses can be contracted from contact with soil, lawn or plants. The antiparasitic history indicated that 81.20% of the dog owners have received antiparasitic drugs in the previous 12 months; while 34% solely rely on pyrantel for activity against nematodes similarly, 54% administered at three-monthly intervals. It was concluded that a high rate of prevalence of gastrointestinal parasites in dogs from different areas found that there were transmission of these gastrointestinal parasites to humans through the faeces of the infected dogs in puppies was alarmingly high than adult dogs.

Keywords: *Toxocara canis; Echinococcus granulosus; Trichuris vulpis; Giardia; dogs*

ICZ-2014-058

**EPIDEMIOLOGY OF DIARRHEA/DYSENTERY CAUSED BY
CAMPYLOBACTER JEJUNI IN CHILDREN OF FAISALABAD, PUNJAB,
PAKISTAN**

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Diarrhea is responsible for one child death out of nine children in the world which makes it the second contributing cause of death in children below the age of five years. Annual death rate due to diarrhea in children under the age of five is 10% worldwide. *Campylobacter jejuni*, a motile, curved S or spiral shaped Gram negative rod, microaerophilic, is a common cause of gastrointestinal infection in infants and children. The present project was designed to study the epidemiology of diarrhea/ dysentery caused by *Campylobacter jejuni* in Children of Faisalabad, Punjab, Pakistan. For this purpose, stool samples of 300 children having age below 60 months were collected from different health centers of Faisalabad (150 of the children having diarrhea and 150 without diarrhea). These samples were evaluated for the presence of *Campylobacter* through specific culture media, staining reaction and biochemical tests. Epidemiological data of each child were collected on a predesigned questionnaire. Data obtained was analyzed through chi-square. Out of 150 diarrheal samples, 30 (20%) *Campylobacter* were isolated. While out of 150 control samples, 3 (2%) *Campylobacter* isolates were obtained. Prevalence of diarrhea was higher in children whose mother's age was below 25 years and the rate decreased with increase in age of children. There was a positive correlation between the prevalence of diarrhea in children and the level of mother's education. Not only attempts must be made to reduce the prevalence of diarrhea but also we need to work on the related factors responsible for diarrhea.

Keywords: *epidemiology; diarrhea; Campylobacter jejuni; children; Faisalabad*

ICZ-2014-059

A STUDY ON TUBERCULOSIS IN PATIENTS OF DIFFERENT SOCIOECONOMIC AND WORK GROUPS IN SELECTED AREAS OF PUNJAB, PAKISTAN

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A study was carried out to look into the socioeconomic factors associated with tuberculosis in Punjab Pakistan. A proforma was specially designed to record the information about each patient and the data obtained was thus analyzed. The sputum samples from smear positive TB cases were collected from different hospitals of Faisalabad, Lahore and Gojra. The pulmonary smear positive TB cases were selected in the study population after informed consent to the patient about the study. The sputum sample of all the suspected patients were screened out after staining with Ziehl Nelsen staining technique. The sputum processing for Ziehl Nelsen staining and PCR was carried out. Total 366 clinical samples of suspected TB patients were collected. All of them were found positive by ZN (Ziehl-Nelsen) staining. Among them, 108 were PCR negative (29.5%; CI 25.00 to 34.34) and 258 were PCR positive (70.49%; CI 65.66 to 75.00). Among these patients, 51.26% were females and 48.74% were males. The frequency distribution showed higher percentage of TB cases in age group of 16-30 years, with body weight 41-50 Kg, in married (75.13%), in uneducated people (54.09%), and those having only up to high school education (41.80%) and in house wives (37.98%).

Keywords: *Tuberculosis; human; Pakistan; PCR; ZN microscopy*

ICZ-2014-060

SEROPREVALENCE OF OVINE BRUCELLOSIS IN DISTRICT THOR GHAR OF KHYBERPUKHTUNKHWA, PAKISTAN

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The study on seroprevalence of brucellosis was carried out on sheep in District Thor Ghar. A total of 500 serum samples and 250 milk samples from sheep were collected and examined with Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Milk Ring Test (MRT). The overall seroprevalence of brucellosis in sheep was recorded as 4%, 3% and 3.33% by RBPT, SAT and MRT respectively. In this study, 250 blood(serum) samples each from male and female sheep were tested. The seroprevalence of brucellosis in females was found as 12%, 8% and 6.66% by RBPT, SAT and MRT respectively, while in males it was observed as 4% and 4% by RBPT and SAT. The seroprevalence of brucellosis in different age groups of sheep was also studied. A total of 500 adult goats, 250 from each males and females, were tested. From 250 adult females (above 12 months age), 12%, 8% and 6.66% were detected as positive by RBPT, SAT and MRT respectively while in 250 young females (upto 9 months age), no brucellosis was detected and considered to be free from brucellosis. A similar number of 250 adult males (above 12 months age) were examined by RBPT and SAT and recorded 4% positive for brucellosis while no brucellosis was recorded by the above techniques in 250 young males (upto 9 months age) and thus considered to be negative for brucellosis. The sera of 250 females were titrated for antibody titer, 4 sera reacted positively with antigen at 1:20, 2 at 1:40 and 2 at 1:80 dilutions; beyond these dilutions no interaction between antigen and serum antibody of positive cases was detected. Similarly antibody titration was carried out against male goats by SAT. Only 2 serum samples were found positive for *Brucella* antibody, from which 1 interacted with antigen at dilution 1:20 and 1 at 1:40. However, beyond this dilution, the interaction between antigen, and antibody was not observed. The incidence of *Brucella* species was also investigated by different techniques. The incidence of *Brucella melitensis* and *Brucella ovis* in the sera of sheep was recorded by RBPT as 6% and 8%, and by SAT as 5% and 6% respectively.

Keywords: *brucellosis; sheep; RBPT; SAT; MRT*

ICZ-2014-061

TARGETED GENE ANALYSIS OF DRUG RESISTANT TUBERCULOSIS IN PATIENTS FROM CENTRAL PUNJAB, PAKISTAN

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The present research work was conducted to study the targeted genes in drug resistant tuberculosis patients from central Punjab, Pakistan. The sputum positive patients were included in the study after informed consent of the patient through specially designed proforma and being screened with Zeihl Neelsen (ZN) staining technique. The obtained data were analysed statistically by applying the chi-square test to check the significance of data. Genetic variation in drug resistant TB patients was evaluated through PCR-RFLP analysis. The results of the present study revealed that 39.42% isolates were found resistant to one or more drugs. Among the drug resistant TB cases, 60.98 % were resistant to Isonizid (INH), 50.41 % to Ethambutol (EMB) and 43.09 % to Streptomycin (STRM). Ofloxacin resistance was noted along with INH, EMB and Streptomycin in 6.5% of resistant cases, hence were declared as XDR-TB cases. We concluded that PCR-RFLP is a very useful molecular technique for the rapid detection of mutations in drug resistant genes of the Mycobacterium from TB patients.

Keywords: *tuberculosis; drug resistance; PCR-RFLP; patients; genetic*

ICZ-2014-062

EPITOPE-BASED IMMUNOINFORMATICS STUDIES OF NUCLEOCAPSID AND G1 GLYCOPROTEIN OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS: GATEWAY TO IMMUNODIAGNOSTICS

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Crimean–Congo Hemorrhagic Fever (CCHF) is an important infectious disease of animals and humans resulting in 50% mortality in humans. The genome of CCHF virus is single stranded RNA which encodes nucleocapsid and membrane glycoproteins. Nucleotide sequence of nucleoprotein and G1 glycoprotein of an asian isolate retrieved from gene bank was used in this study. Nucleoprotein and G1 Glycoprotein were analyzed using different bioinformatic tools. In silico translation and primary structure was done by using Expassy server. Probable secondary structure was predicted. 3D structure was predicted using online homology modeling tool and possible B cell epitopes were predicted using online ABC pred server. Epitope with high ranking score were predicted in nucleoprotein. Present study can eventually be proposed as a base line for diagnostics and development of peptide based vaccine.

Keywords: CCHF; epitope-based immunoinformatics; immunodiagnostic

ICZ-2014-063

OCCUPATIONAL HEALTH AND SAFETY RISK MEASURES CONCERNING ZONOSIS IN THE LIVESTOCK PRODUCTION WORKERS AND FARM ANIMAL WELFARE

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Livestock owners and farm workers rarely could recognize the human health hazards of infectious diseases commonly encountered in most of the livestock operations. Two third of human infectious diseases are reported as zoonotic diseases. Awareness of common risks and knowledge of procedures is needed to reduce the chances of infection and it is key element in preventing zoonotic disease. Taking into account the zoonotic diseases is an important part of a farm safety programme but yet it has not got its prime importance. There is a major threat to the health of people involved in the production process of very food we eat – a terrible consequence of our modern livestock production systems. Zoonosis is a major global threat to public health and as well as animal welfare. Farming methods have changed dramatically in recent decades in order to produce human food in bulk to support drastically increasing human population. As farming methods have become more intensive, there is an increase in animal densities combined with breeding and feeding approaches designed to increase production. It is putting both animals' welfare and human health at risk. It can also lead to serious illness in humans and may be fatal. These changes have a huge impact on farm animals' welfare that we rear for food and can increase the risks of zoonotic diseases as well. These facts are based on different reports written by experts. Some important methodological approaches opted could be ensuring health by developing farming policies that ensure the health of animals and people by better management to minimise stress and optimise animal welfare and immunity, using tools of surveillance and vaccination, investment in research and extension of knowledge regarding transmission of zoonotic infections, helping and supporting farmers to develop and implement standards for livestock welfare, limiting the use of non-therapeutic drugs and reducing the risk of exposure to food infected with infectious agents like Salmonella, Campylobacter and/or *Escherichia coli*.

Keywords: *transmission; zoonosis; Livestock; farming method; animal welfare*

ICZ-2014-064

PRODUCTION AND SOCIO-ECONOMIC FACTORS INFLUENCING THE RISK OF ZONOSIS IN DEVELOPING COUNTRIES

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Present study focuses on pathogen transmission routes, risk factors for disease transmission, pathogens of livestock capable of recombining with organisms in human beings and livestock species that are potential sources of zoonotic infections as well as production and socio-economic factors that influence the risk of transmission, and risk management and control interventions. On the basis of WHO reports there is list of seven endemic diseases that are of zoonotic concern. These include Brucellosis, rabies, human African trypanosomiasis, bovine tuberculosis, cysticercosis, echinococcosis and anthrax. Zoonoses are of concern for two main reasons; the health and economic burdens caused by it. The poor people in developing countries suffer mainly from persisting zoonosis, whilst emerging zoonoses are of more concern for the developed countries. Infectious diseases are responsible for nearly 40-41% of the burden of human sickness and death in low-income countries. Eight percent of this is attributable diseases recently emerged from livestock. In developing countries transmission depends upon contact between livestock, domestic animals and people. Transmission opportunities are subjected to various, sometimes opposing drivers. The root cause of increased transmission opportunities prevailed due to large human population and associated increase in demand for the use of natural resources. These could include the speed of increase in human and animal populations, rapid yet poorly-regulated changes in livestock-keeping systems and close contact between humans and livestock. High livestock numbers, high stocking densities, poor husbandry, and inappropriate use of antibiotics associated with intensification in developing countries all favour disease transmission and emergence. Three important proximal drivers of increased zoonosis are rapidly increasing livestock trade, agricultural expansion, and wet market practices. The socio-economic impact of zoonoses goes far beyond human sickness and death includes multiple burdens such as monetary cost of illness; cost to livestock sector; opportunity cost to economies; negative social and psychological impacts. It can be concluded that developing integrated intervention packages to target risk factors, incorporating technological approaches such as vaccines, population control, disease resistance, biosecurity, husbandry and ecosystem-based interventions. Modelling of different control options (epidemiology, ecology, economics), social and economic innovations, understanding of Key ecological changes for developing countries is important for mitigating zoonosis problem

Keywords: *transmission; disease; socio-economic; livestock; zoonosis; pathogens*