



The 7th International Conference (The 15th Scientific Conference)
“One Health: Animal, Human & Environment: Recent Applications”
Organized by Faculty of Veterinary Medicine, Cairo University, Egypt
Stella Di Mare Sea Club Hotel (Ain Sokhna) August 5 – 8th, 2018



Organizing Committee	
Dr. Eman Fathi	Ass. Professor of Food Hygiene Fac. Vet Med. Cairo Univ.
Dr. Ayman El Deeb	Ass. Professor of Virology Fac. Vet Med. Cairo Univ.
Dr. Alzahraa Abdelatty	Lecturer of Nutrition & Clinical Nutrition Fac. Vet Med. Cairo Univ.
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Dr. Ahmed Orabi	Lecturer of Microbiology Fac. Vet Med. Cairo Univ.
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Dr. Mohamed Ibrahim Shaalan	Lecturer of Pathology Fac. Vet Med. Cairo Univ.



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Scientific Committee	
Prof. Dr. David Hein	Professor, Chair of Pharmacology & Toxicology, School of Medicine, University of Louisville, USA.
Prof. Dr. Kietzmann Manfred	Professor of Toxicology and Pharmacology University of Veterinary Medicine, Hannover, Germany.
Prof. Dr. Babasaheb Sonawane	X- Manager at USA Environmental Protection Agency (EPA) Toxicology and Risk Assessment Consulting Services
Prof. Ayman El-Baz, PhD.	Professor and Chair of Bioengineering Coulter Fellow, University of Louisville, USA.
Prof. Dr. Hany Gohar	Professor of Surgery, anesthesia and radiology Cairo University.
Prof. Dr. Bahgat Edrise	Professor of Nutrition & Clinical Nutrition Cairo University.
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Prof. Dr. Mohamed Zaki	Professor of Poultry Diseases Cairo University.
Prof. Dr. Zakia Ahmid	Professor of Veterinary Hygiene and Management Cairo University.
Prof. Dr. Jakeen El Jakee	Professor of Microbiology Cairo University.
Prof. Dr. Mona El Enbaawy	Professor of Microbiology Cairo University.
Prof. Dr. Maha Hady	Professor of Nutrition & Clinical Nutrition Cairo University.
Prof. Dr. Mohamed El Hady	Professor of Toxicology & Forensic Medicine, Cairo University.
Prof. Dr. Osama El-Tawil	Professor of Toxicology & Forensic Medicine, Cairo University.
Prof. Dr. Khaled El Amry	Professor of Microbiology Cairo University.
Prof. Dr. Eman Baker	Professor of Pathology Cairo University.
Prof. Dr. Hussein Ali Hussein	Professor of Virology Cairo University.
Prof. Dr. Khalid Abdel Aziz	Professor of Zoonoses, Cairo University.
Dr. Ayman El Deeb	Ass. Professor of Virology. Cairo University.



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Sunday	5th August	4.00-5.00 PM	Registration and Opening Ceremony
		5.00-5.05 PM	Peace Republican
		5.05-5.10 PM	Recite Ouraan
		5.10-5.15 PM	Conference Coordinator Speech
		5.15-5.20 PM	Conference General Secretary Speech
		5.20-5.25 PM	Conference Chairman Speech
		5.25-5.35 PM	Veterinary Medicine, Cairo University Overview
		Session 1 (Environmental pollution & Public Health) Chairman: Prof. Dr. Khaled El Amry Prof. Dr. Kietzmann Manfred Prof. Dr. Babasaheb Sonawane) Prof. Dr. Ayman El Baz	
		5.40-6.10 PM	Scientific Presentation entitled "Translating laboratory research to inform human risk assessments following exposures to environmental chemicals." Presenter: Prof. Dr. David Hein
		6.10- 6.20 PM	Discussion
6.20- 6.50 PM	Scientific Presentation entitled "The Bio – impetus of clinico-nutritional strategies on food Safety and public health" Presenter: Prof. Dr. Bahgat Edris		
6.50- 7.00 PM	Discussion		



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Day	Date	Time	Event
Monday	6 th August	Session (2) Virology Chairman: Prof. Dr. Hussein Ali Hussein Prof Dr. David Hein Prof. Dr. Mohamed Zaki Prof. Dr. Eman Baker	
		10.00-10.30 AM	Scientific Presentation entitled "Stress granules as a possible modulator of stem cell fate" Presenter: Prof. Dr. Mohamed Omara
		10.30-10.35 Am	Discussion
		10.35-10.50 Am	Construction and evaluation of recombinant HVT vaccine against Egypt H5 avian influenza strain.
		10.50-11.05 AM	Molecular and serological studies of some strains of RHDV to be compared with vaccine strain.
		11.05-11.20 AM	Genetic variability of haemagglutinin gene of low pathogenic avian influenza virus H9N2 in commercial chicken.
		11.20-11.35 AM	Preparation of trivalent vaccine against lumpy skin disease using different capripox viral strains.
		11.35-11.50 AM	Minor groove binder probe real-time RT-PCR for detection of foot-and mouth disease virus in Egypt
		11.50-12.00 PM	Discussion



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Monday	6 th August	Session (3) Bacteriology Chairman: Prof. Dr. Jakeen Eljakee Prof. Dr. Mona El Enbaawy Prof. Dr. Kietzmann Manfred Prof. Dr. Zakia Ahmid	
		5.00-5.30 PM	Scientific Presentation entitled " Antimicrobials in veterinary medicine: Environmental impact and bacterial resistance." Presenter: Prof. Dr. Kietzmann Manfred
		5.30-5.35 PM	Discussion
		5.35-5.50 PM	Assessment of Some Mycobacterial Antigens in Bovine Tuberculosis Diagnosis.
		5.50-6.05 PM	Serological and molecular detection of subclinical paratuberculosis infection in cattle of dairy herds in Egypt.
		6.05-6.20 PM	Molecular characterization of virulence genes in <i>Campylobacter</i> species in chicken in Egypt.
		6.20-6.35 PM	First report of <i>Bordetella avium</i> in Egyptian turkey flocks.
		6.35-6.50 PM	Molecular studies on some virulence factors of <i>Pseudomonas aeruginosa</i> isolated from chickens as a biofilm forming bacteria.
		6.50-7.00 PM	Discussion
		Posters (will be displayed during the whole session)	-Investigation of Contagious Bovine Pleuropneumonia vaccination in Khartoum State, Sudan 2016-2017 -Molecular Characterization of <i>Riemerella anatipestifer</i> Isolated from Ducks' Outbreaks in Egypt



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Day	Date	Time	Event
Tuesday	7 th August	Session (4) Recent Medical & Research Approaches Chairman: Prof. Dr. Osama El-Tawil Prof. Dr. Babasaheb R Sonawane Prof. Dr. Ayman El-Baz Prof. Dr. Bahgat Edrise	
		10.00-10.30 AM	Scientific Presentation entitled " Electronic Waste Exposure an Evolving Global Public Health and Environmental Threat: Risk Assessment Challenge" Presenter: Prof. Dr. Bob Sanawane
		10.30-10.35 AM	Discussion
		10.35-11.05 AM	Scientific Presentation entitled "Role of Big Data in Personalized Medicine" Presenter: Prof. Dr. Ayman Elbaz
		11.05-11.10 AM	Discussion
		11.10-11.25 AM	Silver nano-particles and their antibacterial activity, histopathological effects in rainbow trout (<i>Oncorhynchus mykiss</i>).
		11.25-11.40 AM	Regulation of Muscle Mass growth via Microinjection of CRISPR/Cas9 Protein into Channel Catfish, <i>Ictalurus punctatus</i> , One-cell Embryos targeting Myostatin Gene
		11.40-11.55 AM	Pharmacokinetics/ Pharmacodynamics of intramammary cefquinome in lactating goats with and without experimentally-induced <i>Staphylococcus aureus</i> mastitis.
		11.55-12.00 AM	Discussion



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Day	Date	Time	Event
Tuesday	7 th August	Session (5) Multidisciplinary session Chairman: Prof. Dr. Hany Gohar Prof. Dr. Hussein Omar Prof. Dr. Mohamed El Hady Prof. Dr. Maha Hady	
		5.00-5.15 PM	Developmental Interventions for Biorisk Management in Livestock Sector- the Dairy Science Park Approach. Prof. Dr. Muhammad Kakar
		5.15-5.30 PM	Food hazards “Permissible limits in Food.
		5.30-5.45 PM	Biological activity and physicochemical quality of different types of kombucha yoghurt VS traditional yoghurt during storage.
		5.45-6.00 PM	Evaluation of subcutaneous infiltration of autologous platelet-rich plasma on skin-wound healing in dogs.
		6.00-6.15 PM	Prevalence of Intestinal Coccidiosis and the Associated Economic Losses among Sheep and Goats in Bahrain.
		Posters	Liver histopathology in camels (<i>Camelus dromedarius</i>) fascioliasis in Sudan.
		6.15-7.00 PM	Closing Ceremony & Recommendations



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Translating laboratory research to inform human risk assessments following exposures to environmental chemicals

David W. Hein

Department of Pharmacology & Toxicology and James Graham Brown Cancer Center, University of Louisville, Louisville, Kentucky USA

Research findings from the laboratory have improved the understanding of arylamine carcinogen metabolism leading to improved design and interpretation of human molecular epidemiology investigations. Laboratory studies that infer and test biological plausibility, including cancer risks modified by differential metabolism of arylamine carcinogens in rapid and slow arylamine *N*-acetyltransferase (NAT2) acetylators, have been critical for investigating the role of smoking in the etiology of human cancers. These concepts will be illustrated with laboratory research derived from genetically engineered animal models and cell lines, and human cryopreserved hepatocytes. Research findings inform urinary bladder cancer risk assessments, in which the role of tobacco smoking is now established and breast cancer where a consensus for the role of tobacco smoking remains a work in progress.

Keywords: Arylamine carcinogen metabolism; Smoking cancer; Risk assessments.

Biography

Dr. Hein serves as Peter K. Knoefel Endowed Chair of Pharmacology, Professor and Chairman of the Department of Pharmacology & Toxicology, and Distinguished University Scholar at the University of Louisville. He leads four National Institutes of Health-funded training programs: University of Louisville Cancer Education Program funded by the National Cancer Institute; University of Louisville pre- and post-doctoral training program in environmental health sciences funded by the National Institutes of



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Environmental Health Sciences (NIEHS); University of Louisville Superfund Research Center Training Core funded by NIEHS; and University of Louisville Hepatobiology and

Toxicology COBRE Faculty Career Development Program funded by the National Institute of General Medical Sciences. He has served as reviewer of grant proposals for the National Institutes of Health and other funding agencies and a consultant to numerous companies across the USA and the world. His research program includes studies of the molecular epidemiology of cancer susceptibility, pharmacogenetics, genomics, personalized medicine, and functional genomics. He has coauthored about 250 peer-reviewed journal articles and book chapters, 75 published gene sequences, and over 600 abstracts. The publications have over 13,500 citations with an h-index 59. He has served as principal investigator/co-investigator/mentor on over 75 research grants and contracts totaling over \$50 million dollars. Further info is available at <http://louisville.edu/faculty/dwhein01>.



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The Bio – impetus of clinico – nutritional strategies on food safety and public health

Prof . Dr . Bahgat Moustafa Edrise

M.V.Sc ; M.D ; F.R.C.V.S ; Ph.D. Dip . Ed. Tech; Master & Doc (in Med. Educn. / Healthcare Management & Leadership) (EGYPT, U.K, USA)

The worry list of food – safety fears is progressively growing world – wide during the last two decades. The global aims of food producers and manufacturers are to introduce to the human "Food Chain" top quality, safe foods of high nutritive value and health – potentials / credentials matching the "Codex Alimentarius" specifications.

Food – safety is a vital Public Health concern that encompasses and warrants customization of " Pre – requisite Programs ". The Bio – impetus of "Clinico – nutritional strategies on food safety and Public Health "have shown evidence - based significant impact during the recent years.

Keywords; Food – safety; Public Health; Clinico – nutritional strategies; Health credentials.

Biography

Dr. Bahgat Moustafa Edrise

Prof of Clinical Nutrition (UK- Cairo University).

Adj. Prof. of Healthcare Quality Management

(Arab Acad. Sci & Technology).

Master & Doc degrees in Med. Education & Healthcare Quality Management (BU & HARVARD -USA).

INTERNIST Fellow (UK).

Intl. Certified Trainer (USA).

Intl. Certified Hospital Surveyor (OKLAHOMA -USA)



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Stress granules: A potential hub in regulating stem cell fate.

Mohamed M. Emara^{1,3*}, Freshteh Palangi¹, Samson M. Samuel², Ian R. Thompson¹, Chris R. Triggle²

¹ Neurological Disorders Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Qatar Foundation, Doha, Qatar.

² Weill Cornell Medical College-Qatar, Qatar Foundation, Doha, Qatar

³ Department of Virology, Faculty of Veterinary Medicine, Cairo University

Stress Granules (SGs) are dynamic ribonucleoprotein aggregates, which have been observed in cells subjected to environmental stresses, such as oxidative stress and heat shock (HS). Pluripotent stem cells (PSCs) are highly sensitive to oxidative stress, indicating the importance of SGs in regulating stem cell fate. In this study we compared the effects of oxidative (sodium arsenite (SA) and hydrogen peroxide (H₂O₂)) and thermal HS stressors on SG formation in human induced (hi) PSCs. The aim was to establish whether these granules have a role in regulating PSC self-renewal and differentiation. We found that SA and HS, but not H₂O₂, induce SG formation in hiPSCs. The analyses of these granules showed that they are canonical SGs, because (i) they contain the well-known SGs proteins (G3BP, TIAR, eIF4E, eIF4A, eIF3B, eIF4G, and PABP), (ii) they were found in juxtaposition to processing bodies (PBs), and (iii) they were disassembled after the removal of the stress. Consistent with the SG data, SA and HS, but not H₂O₂, promote eIF2 α phosphorylation in hiPSCs forming SGs. An initial screening for pluripotent marker proteins recruited to SGs confirmed that LIN28A and L1TD1 were SG markers and identified DPPA5 as a novel pluripotent marker that was weakly recruited to SGs. Altogether, our data introduce new aspects of how hiPSCs respond to adverse environmental conditions.

Keywords: Environmental stresses; Stem cells; Stress Granules.



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Biography

Dr. Mohamed Emara is an Assistant Professor at the virology department, Faculty of Veterinary Medicine, Cairo University (leave of absence). Currently he holds a scientist position at the Qatar Biomedical Research Institute (QBRI) and an Assistant Professor at Hamad Bin Khalifa University, Doha, Qatar. Dr. Emara graduated from the Faculty of Veterinary Medicine, Cairo University in 1995, where he was appointed as a demonstrator at the virology department. In 1999, he finished his Masters in molecular biology of viruses and then traveled to the US to finish his PhD studies. He received a PhD in Molecular Genetics and Biochemistry from Georgia State University in 2007. Subsequently, he was a Research Fellow in medicine at Harvard Medical School where he spent four successive years studying the different components of the stress response pathway and their mechanism in regulating the stress response program. Beside his research and teaching duties, Dr. Emara has several administrative activities where he was the first scientist to join QBRI where he actively participated in the establishment of the institute centers with special focus on the stem cell platform and iPSC core facility. In addition he currently serves as the institute and the university IRB coordinator. Dr. Emara main research focus is directed towards using human induced pluripotent stem cells (hiPSCs) in neurological diseases modeling with a special interest on neurodevelopmental (ASD) and neurodegenerative diseases (PD). Dr. Emara's lab utilizes the stem cell based approach to generate hiPSCs from patients and healthy individuals and differentiate them into neurons. Functional neurons will be used as a cell model to study the molecular and cellular mechanisms behind the disease pathogenesis and to perform

transcriptomic, epigenetic, and functional analyses to help us to identifying putative biomarkers for disease diagnosis. Another branch the Dr. Emara's lab cover is the understanding the possible role of stress response program components in regulating stem cell self-renewal and differentiation, with a special focus on neuronal differentiation.



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Antimicrobials in veterinary medicine:

Environmental impact and bacterial resistance

Manfred Kietzmann

Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine Hannover, Foundation, Bünteweg 17, D-30559 Hannover, Germany

Antimicrobials belong to the most successful therapeutic agents for treating infectious diseases in human beings and animals. Already Alexander Fleming who detected the antibacterial activity of penicillin described the problem of a developing bacterial resistance against antimicrobials. Today we know very well that this is unfortunately the truth. As director of the U.S. Centers for Disease Control and Prevention, Thomas Frieden mentioned in 2013 the bacterial resistance as one of the most serious health threats. “The loss of effective antibiotics will undermine our ability to fight infectious diseases and manage the infectious complications common in vulnerable patients.” Considering the one health initiative, various action schedules were initiated in human and veterinary medicine worldwide to avoid such a post-antibiotic era.

In farm animals, antimicrobials (mainly administered orally) have significantly improved the health status. However, the increasing use of antimicrobials in recent decades in livestock production gives cause for several concerns. First, the chronic and often massive exposure of the animals to antimicrobials includes a certain amount of risk regarding selection of resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) or extended spectrum β -lactamase (ESBL)-producing bacteria and can promote the development of resistance reservoirs. Secondly, exhaust air (including dust) and manure from housed stock treated with antimicrobials can carry considerable amounts of antibiotic substances and resistant microorganisms into the direct environment and to nearby residents or neighbouring farms. Third, after treatment



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residues of antimicrobials in products like meat, eggs and milk may reach the consumer. Various guidelines exist which should ensure a prudent use of antimicrobials including criteria for the selection of most appropriate antibiotic preparations. The prudent use of antimicrobials in veterinary medicine as well as in human medicine is necessary to ensure their efficacy in the future. Further activities are important to handle the risk of bacterial resistance in human beings, animals and in the environment (one health). Antimicrobials are bound for therapeutic use as medicinal compounds (including metaphylaxis) in cases of diagnosed bacterial infections in human beings and animals. Whenever these drugs are used it has to be considered that a significant amount of the compounds itself or their metabolites are reaching the environment with the already mentioned consequences of an environmental contamination and boost of the bacterial resistance.

Keywords: Antimicrobials; Bacterial resistance; Methicillin-resistant *S. aureus*.

Biography

Department of Pharmacology, Toxicology and Pharmacy University of Veterinary Medicine Hannover, Foundation Bünteweg 17, D-30559 Hannover

E-Mail: manfred.kietzmann@tiho-hannover.de

Born in Meschede (Germany) 1954

Studies of Veterinary Medicine (Hannover) 1974-1979

Dissertation (Pharmacokinetics of sulphonamides in chicken, Hannover) 1980

Assistant at the Dept. of Pharmacology, Toxicology and Pharmacy

University of Veterinary Medicine Hannover 1980-1994

Habilitation (Pharmacological effects on epidermal proliferation

and differentiation, Hannover) 1993

Diplomate of the European College of Veterinary Pharmacology and Toxicology 2005

Professor for Toxicology, Institute for Pharmacology, Pharmacy and Toxicology

Faculty of Veterinary Medicine, University of Leipzig 1994-1997

Professor for Toxicology and Pharmacology, Department of Pharmacology,

Toxicology and Pharmacy, University of Veterinary Medicine Hannover since 1997



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Electronic Waste Exposure an Evolving Global Public Health and Environmental Threat: Risk Assessment Challenge.

Babasaheb (Bob) Sonawane¹; Jayaram Kancherla² & Bruce A. Fowler¹

¹Toxicology and Risk Assessment Consulting Services, LLC, North Potomac, Maryland (USA)

²University of Maryland, College Park, Maryland,(USA).

Recently, an increasing demand and dependence on electronics has caused rapid accumulation of electronic waste (e-waste) around the world. An estimated over 65 million tons of e-waste was created globally in 2017, with significant increases projected in the years ahead. E-waste is composed of combination of several hazardous substances such as metals, metallic compounds, and organic chemicals. Direct or indirect exposure (soil, air, drinking water food) to these substances are a threat to the environment, animal and human health, especially to vulnerable populations such as children and pregnant women. Studies of adverse health outcomes in humans related to e-waste exposure have shown increase in spontaneous abortions; stillbirths, premature and reduced birth weights and birth lengths. For economic reasons, recycling and reclamation of certain precious elements such as gold, copper, indium and gallium are frequently performed in less developed countries by workers using primitive techniques such as open burning, with little or no safeguards in place to protect for human and environmental health.

This presentation will focus on the scope, nature, and magnitude of global e-waste problem, global distribution streams, and differences in handling e-waste between developing and developed countries, occupational and environmental exposure issues and human health effects, including populations at special risk. Discussion will also focus on inorganic and organic chemicals and how the combination of e-waste chemical mixtures and other socio- economic stressors present a unique and important challenge for human health and environmental risk assessment. Furthermore, it will briefly cover the translation of risk and risk communication to public at-large and including the potential



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role of decision -makers at national and international levels to develop effective policies and implementation strategies that could be undertaken to reduce exposure burden to e-waste. We will also identify data gaps and research needs to better characterize health risk of e-waste materials including suggestions for reducing their risk.

Keywords: Electronic waste (e-waste); Hazardous substances; Human health; Health risk.

Biography

Dr. Sonawane received awarded PhD degree in Entomology by the University of Missouri, Columbia Campus in 1971. He was a NIH postdoctoral Fellow at the National Institute of Environmental Health Sciences from 1972-1975. He served as a faculty member in the Departments of Pediatrics at the Children’s Hospital of Philadelphia, the School of Medicine, as well as at the School of Veterinary Medicine of the University of Pennsylvania, Philadelphia, PA from 1975- 1983. He worked with the U.S. Food and Drug Administration in Rockville, MD and the U. S. Environmental protection Agency in Washington, DC in various capacities He is very well recognized expert in the fields of Toxicology and Risk Assessment of chemicals. He is an author and/or co-author of over 100 publications and several book chapters in the fields of toxicology and pharmacology, children’s environmental health and risk assessment. In 2016, he was awarded the most Distinguished Service Citation by the Administrator of USEPA in recognition of his dedicated service to the American People and abroad. Dr Sonawane was honored to serve as an invited member of the Nomination Committee for the Heinz Foundation Awards (2011-2013).

Dr Sonawane initiated, planned, organized, sponsored and/or participated in numerous scientific workshops, meetings and conferences actively promoting environmental health and risk assessment issues and challenges of environmental agents in several countries around the world. Dr. Sonawane throughout his professional career served as a mentor and advisor and has trained 40 plus scientists at different level of their career.



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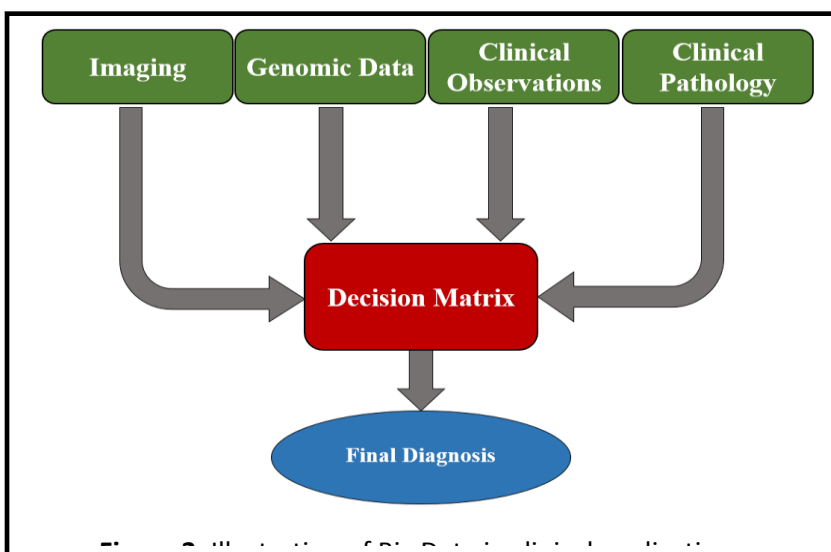
Role of Big Data in Personalized Medicine

El-Baz, Ayman

Professor and Chair of Bioengineering Coulter Fellow, University Scholar

Speed School of Engineering University of Louisville

The term “Big Data” refers to the amalgamation and processing of huge data sets that are composed of different data types (e.g. clinical, genomic, imaging, pathological, etc.) and have rapidly become more massive and complex, particularly with the advent of new technologies. Big



Data within the context of biomedical research is a major problem that needs to be solved due to substantial increases in the amount of medical data routinely generated and collected by healthcare providers over the last two decades. Recent PubMed search for the term “big data” yields 1470 entries, with the earliest occurring in 2003. A breakdown by year shows the majority of publications are from 2012 or later (Figure 1). In 2011, the McKinsey Global Institute issued a 156-page report titled “Big data: The next frontier for innovation, competition, and productivity”. This report indicated \$300 billion in potential annual value in Big Data to health care in the U. S., with a shortage of 140,000 to 190,000 individuals with the required deep analytical skills, indicating a need for programs to train the next generation of scientists with the necessary skill set to deal with all aspects of Big Data.



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The current main challenge is that our ability to advance medical care and efficiently translate science into modern medicine is bounded by our capacity to process and understand these big data. So, there is an urgent need to develop and integrate new statistical, mathematical, visualization, and computational models with the ability to analyze Big Data in order to retrieve useful information to aid clinicians in accurately diagnosing and treating patients to improve patient outcomes. Thus, the main objective of this talk is to give an overview of the new computational models and the state-of-the-art machine learning approaches to analyze and integrate multiple data types for the creation of a decision matrix that aids clinicians in the early diagnosis and identification of high-risk patients for human diseases and disorders such as Autism.

Keywords: New technologies; Big Data; Early diagnosis.

Biography

Dr. Ayman S. El-Baz is a Professor, University Scholar, and Chair of the Bioengineering Department at the University of Louisville, KY. Dr. El-Baz earned his doctoral degree in electrical engineering from the University of Louisville in 2006. In 2009, Dr. El-Baz was named a Coulter Fellow for his contributions to the field of biomedical translational research. Dr. El-Baz has 15 years of hands-on experience in the fields of bio-imaging modeling and non-invasive computer-assisted diagnosis systems. He has authored or coauthored more than 450 technical articles (105 journals, 15 books, 50 book chapters, 175 refereed-conference papers, 100 abstracts, and 15 US patents).



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Sequence analysis of seven equine herpes type 1 viruses circulating in non- vaccinated arabian and foreign horses in Egypt

**Nagwa Khaled¹; Abo El-khair M¹; Ahmed BM²; El-Soally S³; Abd el-Hamid MA⁴; Nayel M⁵
Hussein HA^{2*}**

¹ Department of Virology,⁴ Department of Veterinary Pathology, ⁵ Department of Infectious Diseases, Faculty of Veterinary Medicine, University of Sadat City, 32511, Menoufiya, Egypt, ³ Military Veterinary Hospital, Egyptian Armed Forces, Cairo, Egypt, ² Department of Virology, Faculty of Veterinary Medicine, Cairo University, 12211, Cairo, Egypt

Equine herpesvirus 1 (EHV-1) has a significant economic impact on equine industry. It is a highly contagious pathogen, mainly transmitted by inhalation of aerosols of virus laden respiratory secretions especially with high population during equestrian events. Reactivation of virus in non-symptomatic, latently (silently) infected horses is the main cause of circulation of such virus in horses. In the present study, molecular characterization of circulating EHV-1 viruses among horses of equestrian clubs in Egypt was carried out. Sixty-two samples of whole blood with anticoagulant were collected and screened for EHV-1 using nested PCR that amplify the conserved fragment of glycoprotein B gene (ORF 33), followed by sequencing and phylogenetic analysis. The study revealed that 19% of our samples were positive to EHV-1 and phylogenetic analysis demonstrated that the Egyptian EHV-1 isolates were more than 99% similarity to European abortogenic isolates (EHV-1: strains 183 and Suffolk/48/2013). Indeed, the analysis reports that these viruses are circulation in both Arabian and foreign horses in Egypt. Control strategy for these viruses in vaccinated and non-vaccinated need to be addressed.

Keywords: Nested PCR; Equine herpes virus type 1 (EHV-1); Glycoprotein B gene (gB, ORF 33); Horse; Egypt.



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Organized by Faculty of Veterinary Medicine, Cairo University, Egypt
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Preliminary studies on preparation and evaluation of a local isolate tissue culture propagated pigeon pox vaccine

Kafafy, M. H¹, Aboul-Soud, E. A. ¹, Olfat E. Nakhla¹

Christin. A. Michael¹, and Nermin. M. El-Said²

¹Veterinary Serum and Vaccine Research Institute (VSVRI),
El-Seka-Beda Street, Abbasia, 131, Cairo, Egypt

²Central Laboratory for Evaluation of Veterinary Biologics (CLEVB)

The current study was conducted to use the propagated Egyptian strain of pigeon pox Qaluobeia 2017 virus on primary chicken embryo fibroblast (CEF) in preparation of tissue culture (VERO) specific pigeon pox virus vaccine (PPV) for pigeons. The titer of this virus was $10^{5.5}$ TCID₅₀ /ml at the 20th passage, and induced an acceptable reaction when it was tested for pathogenicity in pigeons. After addition of an equal volume of Lactalbumin sucrose stabilizer the virus fluid was lyophilized showing titer of $10^{5.0}$ TCID₅₀/ml in VERO cells. Keeping quality control tests revealed that such vaccine was free from the foreign contaminants, safe and potent. The humoral antibody level in the serum of vaccinated pigeons was measured by serum neutralization test (SNT) which proved that the induced pigeon pox antibodies had 1.3 neutralizing index (NI) from the 2nd week and reached its peak (2.8 NI) at 4th week post vaccination. Pigeons withstand the inoculation with the virulent PPV after 3 weeks from vaccination with no symptoms of PPV disease on contrast to control non- vaccinated pigeons with protection percent 95%. This study recorded the production of a safe and potent PPVV from local PPV strain (Qaluobeia -2017) of less cost than that prepared on specific pathogenic free embryonated chicken egg (SPF-ECE)

Keywords: Pigeon pox; Vaccine; Serum neutralization test.



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Lyophilization as an alternative method for preservation of some continuous cells cultures

Shendy, M. B; EL-Dakhly, A. T; Effat, L. El Sayed; Albehwar, A. M; Abu-Elnaga, H. I.

Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt. P.o. Pox 131

This work is a trial to provide lyophilization as a simple method for preservation of continuous cell lines instead of the freezing process in liquid nitrogen. Comparative evaluation of lyophilized African green monkey kidney (VERO), baby hamster kidney (BHK) and Madin Darby bovine kidney (MDBK) cell lines and those preserved by freezing in liquid nitrogen was carried out. Such evaluation on 6 monthly intervals revealed that both of lyophilized cell cultures showed delayed cell adhesion to the culture surface extending to 2-3 days post culturing while propagated and frozen cells adhered to the culture surface within few hours. However there were an increased number of adhered viable cells in case of loading of cells by trehalose or sucrose on days post culturing. In addition cultured lyophilized cells which loaded by trehalose or sucrose exhibited abnormal shape (showing cell rounding) in comparison to other cultures. Also cell dispersing of confluent sheets of cultured lyophilized cells found to be longer (one hour) than that required to other cultures (few minutes) even on using EDTA and incubation at 37°C. Further studies are needed to investigate the biological behavior or cell changes which may be occurred through the lyophilization process in addition to study the ability of such cells to virus infection. It also could be suggested that preservation of cell lines by lyophilization may be of value in cell culturing suspension system.

Keywords: Lyophilization; Preservation; Cells cultures.



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Construction and evaluation of recombinant HVT vaccine against Egypt H5 avian influenza strain

Azab. A. Ahmed^{1,2}, Pucheng Chen², Jinxiong Liu², Abdelsttar Arafa¹ and Hualan Chen²

¹Reference laboratory for veterinary quality control on poultry production, AHRI, Egypt. ²State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, CAAS, People's Republic of China

H5N1 avian influenza virus was endemic in Egypt. Long term circulation of HPAIV resulted in the evolution of new clusters. AI vaccines are considered a suitable tool to support AI control programs in combination with other control measures such as good biosecurity and monitoring programs. HVT is widely used as a live vaccine against Marek's disease and also, is being used as a vector for development of recombinant vaccine. Here, we first established a system to generate the HVT vaccine strain by using the transfection of overlapping fosmid DNAs. Using this system, we constructed recombinant virus, rHVT-ul53HA, in which the hemagglutinin (HA) gene of the H5N1 virus A/chicken/Egypt/75S/2015 was inserted and stably maintained within the ul53 gene of the HVT genome. Recombinant HVT viruses were used to infect chicken embryo fibroblasts. Plaques and the growth kinetics of rHVT-UL53-HA-infected chicken embryo fibroblasts were similar to those of parental HVT. Confirmation of the expression of the H5 HA gene in cells infected with the rHVT-H5HA has been done by immunofluorescence. Further chicken trial is still under study.

Keywords: H5N1; Vaccine; Hemagglutinin gene; Immunofluorescence,



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Epidemiological and phylogenetic analysis of Newcastle disease virus circulating in poultry farms in Egypt during 2015-2016

Naglaa Hagag, Ali Zanaty, Neveen Rabie, Mahmoud Saied, Kareem Selim, Azhar Gaber, Abdel-Sattar Arafa, Mohamed Khalifa Hassan

Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt.

Newcastle disease virus (NDV) is a highly contagious disease in poultry, also considered as a major challenge for the commercial and traditional poultry industry in Egypt, even in vaccinated flocks. A total of 3700 different samples (organs, swabs and isolates) that represented 335 suspected flocks were collected from suspected NDV infected poultry farms showing respiratory manifestation and/or drop in egg production during 2015-2016. Only 56 NDV outbreaks were confirmed in different chicken farms by RRT-PCR, with prevalence rate 16.7%. The NDV outbreaks were recorded in 14 governorates from total 19 investigated governorates and the recorded geo-prevalence of 73.7 %. Twenty five samples were selected for further sequencing for the partial fusion protein. Phylogenetic analysis revealed that 20 samples are genotyped as very virulent NDV class II of genotype VIIb, 4 samples were of high identity (94%-100%) with NDV class II of genotype II (vaccine strain) and 1 sample was phylogenetically related to NDV class II of genotype I with 98% identity with vaccine strains. Sub-genotyping phylogenetic analysis of genotype VII revealed that all the virulent viruses belonged to genotype VIIb.

Key words: NDV; RRT-PCR; Fusion gene (F gene); Phylogenetic analysis.



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Molecular and serological studies of some strains of RHDV to be compared with vaccine strain.

Dalia, A.M. Abd El-Moaty¹, Samah, E.A Abo-Dalal², Owees, .G.A. Salman², Nabil, Abdel-Wanees², Ashraf M. Abbas¹.

¹Genetic Engineering Research Dep., Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt. ² Department of poultry vaccines, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt.

Vaccination is the major control measure for RHDV. The circulation of both classical and variant RHDV subtypes in Egypt evoked the need to determine the most effective vaccine strain and the cross protection between these subtypes. Sixty cross breed and Bosket commercial 3 months susceptible adult rabbits were vaccinated with commercial variant vaccine (Giza2006) and classical strain vaccine (Giza97). Rabbits were challenged three weeks post vaccination with variant RHDV strains (Giza2010 & Kal2012) and original RHDV (Giza97& Kal2014) to determine the cross protection and evaluate the immunity and cross reactivity by HI and indirect ELISA. Both vaccines were protective with higher percentage for variant vaccine (83.4%) than classical vaccine (81.25%) without direct relation between mortalities and the genotype of the challenge strain. The antibody titers using HI test showed 1 log higher for variant over the classical vaccines, but post challenge titers showed increased reactions expressed with 2-3 log higher for classical vaccine. Sequence and phylogenetic analysis of RHDV2014 revealed its relatedness to classical RHDV genotype without any evidence to the presence of RHDV2 in the Egyptian field to that date. In Conclusion, both classical and variant based vaccines are protective against both relevant RHDV genotypes but continuous monitoring of circulating RHDV as the emergence of RHDV2 in the Egyptian field will require new vaccination strategies.

Keywords: RHDV; Vaccine, ELISA; Phylogenetic analysis.



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Genetic variability of hemagglutinin gene of low pathogenic avian influenza virus H9N2 in commercial chicken

**Amany Adel, Wesam Hassan, Zienab Mosad, Fatema Amer, Asmaa Shaban, Marwa Ali,
Abdel-Satar Arafa, Mohamed Kamal, Mohamed Khalifa**

Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Giza 12618, Egypt

Since the first isolation of the H9N2 low pathogenic avian influenza virus in 2011, the virus has distributed rapidly and widely in different poultry sectors in Egypt causing a severe economic losses and problematic situation in poultry production especially with a co-infection with other circulating pathogens. In this study, a total 23182 suspected cases of collective cloacal and tracheal samples were received during 2015-2016 in Reference laboratory of veterinary quality control on poultry production (RLQP) from different poultry sectors and species which are distributing all over the Egyptian governorates for examination of LPAI (H9N2) virus by real time RT-PCR, resulted in 1026 confirmed positive cases for H9N2 with prevalence rate 4.4%. However, the LPAI H9N2 showed a wide range distribution with high geo-prevalence rate in 2015/2016 (96.3%) as positive cases were recorded in 26 governorates. Totally, the positive samples were distributed in 783 farms with the highest prevalence rate (76.5%), then 167 LBM (16.5%) and 76 households (7%), respectively. Also, the most of positive cases were detected in chicken as the highest prevalence (90%) for the H9N2 infection among all the examined species, followed by Turkey (4%), duck (2.6%) and quail (2.4%), respectively. Genetically, the genetic sequence for the hemagglutinin (HA) of 44 recently circulating Egyptian viruses are belonging to the Middle East G1- like viruses that are closely related to each other and scattered phylogenetically in different subgroups with the presence of variant viruses in the quail during 2015.

Keywords: Hemagglutinin (HA); LPAI-H9N2; G1-LIKE; Phylogenetic; RT-PCR; Mutations analysis; RNA.



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Preparation of trivalent vaccine against lumpy skin disease using different capripox viral strain

Kafafy, M. H.¹, El Soally, S.A.², Aboul-Soud, E. A.¹, Zaghoul, M.A.³ and Christine A. Mikhael²

¹Veterinary serum and Vaccine Research Institute, Pox department, Abbasia, Cairo. ²Military Veterinary Hospital. Cairo. ³Central Laboratory for Evaluation of Veterinary Biologics

In Egypt since the appearance of LSD in 1988, various live attenuated sheep pox vaccines were used. Because the Lumpy Skin Disease (LSD), became endemic in Egypt, the aim of this study was preparation and evaluation a new live attenuated tissue culture trivalent vaccine from two sheep pox viruses (Romanian, Kenyan sheep pox) and one goat pox viruses (Held goat pox virus) as a trial to improve vaccination against LSD specially when we know that there is across immunity between them. The previous Capripox viruses were titrated separately in Vero cell, and were mixed with each other in equal volumes (1:1:1) with equal titers ($4.5 \log_{10} \text{TCID}_{50}/\text{ml}$). The experiment applied on 24 calves of 6-12 months, they were divided into two groups (12/ group); the first group was vaccinated with a new trivalent vaccine. The second group was inoculated with available produced monovalent Romanian sheep pox vaccine. And 8 animals were left as non-inoculated contact controls. The cellular immunity of all animals was estimated using lymphocyte blastogenesis measured by XTT assay and the humoral immunity were evaluated by serum neutralization test and ELISA. The results revealed that the trivalent vaccine showed high level of cellular immunity for long time than the single Romanian sheep pox vaccine. Also the level of neutralizing antibodies by SNT and ELISA remained protective in animals vaccinated with trivalent vaccine. A Challenge test was applied using 0.5 ml of virulent LSDV ($5 \log_{10} \text{TCID}_{50}/\text{ml}$) every 3 months till the end of experiment (12 months post inoculation). In conclusion the current study proved that a trivalent vaccine was safe, potent, high immunogenic and provide long duration of immunity.

Keywords: the Lumpy Skin Disease; Sheep pox virus; lymphocyte blastogenesis; ELISA.



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Minor groove binder probe real-time RT-PCR for detection of foot-and mouth disease virus in Egypt

Hany I Abu-Elnaga

Department of Foot and Mouth Disease, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt PO Box 131. Postal No.11381.

Shorter, more specific minor groove binders (MGBs) probes are dsDNA-binding agents attached to the 3' end of TaqMan probes that could be designed strictly to invariant region. Application and assessing of a new trend for viral detection in Egypt depending on MGB probe real-time RT-PCR (rRT-PCR) was applied on local FMDV serotypes O, A, and SAT2. Moreover, FMDV O were detected using two serotype specific primer sets by SYBR Green real-time RT-PCR assaying rapid formats. The limit of detection of diluted RNAs using MGB probe rRT-PCR assay reached to ≤ 6 fg / ul. Besides, high specificity of the former assay was clear. In contrary, the employing of FMDV O specific primer pairs in SYBR Green real-time RT-PCR showed a relative less sensitivity and specificity, particularly one of them displayed a very poor performance. Lastly, the local financial cost of MGB probe is considered the obvious hinder.

Keywords: Foot-and-Mouth disease virus; rRT-PCR; MGB probe.



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Studies on the using of 2-phenoxyethanol as an alternative to thiomersal as a preservative in Foot-and-Mouth Disease vaccine

Hany I Abu-Elnaga, Sonia A Rizk, Hind M Daoud, Akram Z Hegazy and Walaa S Shabana

Department of Foot and Mouth Disease, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt.

The progress in foot-and-mouth disease (FMD) vaccine production was primarily directed towards the safety of the vaccine, purity of the antigen, selection of proper additives, as adjuvant and preservative. Thimerosal (Merthiolate) has been used as a preservative since the 1930. Nevertheless, it is important to note that thiomersal itself can prove to be very toxic because it contains mercury. Hence, the current article discussed the cause and the prevention measures of the pyrogen-free colored sediment that might appear in the vaccine formula. Where, the etiology of the sediment that might appear in the biological product was approached and solved. Besides, 2-phenoxyethanol was examined as an alternative preservative in FMD vaccine, where it showed safety and efficacy as substitutional.

Keywords: Foot-and-Mouth disease virus; Thiomersal; 2-phenoxyethanol.



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IMS 1313-Nanoparticle Mucosal Vaccine Enhances Immunity against Avian Influenza and Newcastle Disease Viruses

**Nermeen M. Ismail¹, Ayman H. El-Deeb², Mohamed M. Emar², Hoda I. Tawfik¹, Nabil
Abdel Wanis¹, Hussein A. Hussein^{*2}**

¹Department of Newcastle Disease, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo.

²Department of Virology, Faculty of Veterinary Medicine, Cairo University.

This study aimed to develop efficacious vaccines and vaccination strategies against avian influenza and newcastle disease viruses. Two formulations of bivalent vaccines for Avian Influenza and Newcastle disease viruses were prepared based on the use of IMS1313 nanoparticles (mucosal vaccine) and Montanide ISA71 (parental vaccine) adjuvants. The prepared vaccines were delivered in specific pathogen free (SPF) chickens with different vaccination protocols. Cell mediated and humoral immune response (cytokine expression levels including IFN- γ and IL6; lymphocyte proliferation; antibodies titers against both H5N1 and NDV) were measured. Challenge trial was carried out to determine the protection percent and shedding pattern of the challenged viruses. Our results revealed a significant increase of IFN- γ and IL-6 genes expression and lymphocytes proliferation in the vaccinated groups compared to the unvaccinated group. Two applications of the mucosal vaccine demonstrated higher HI titers and protection percent ranged from 40% – 50% with different levels of virus shedding as measured by qRT-PCR assay. However, when the vaccines were applied in a prime-boost protocol (mucosal-parental; respectively), protection reached 90% and 100% against AIV and NDV, respectively. No shedding of the NDV-challenge virus was detected whereas, AIV-challenge virus was detected in the samples of the 3rd day post-challenge. Indeed, the use of mucosal-parental vaccines in a prime-boost vaccination protocol demonstrated the potentiality of such approach.

Keywords: ISA71; IMS1313; NDV genotype VIIId; H5N1.



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Antigenic content of 500 HA units in H5N1 reassortant AI vaccine enhance protection and reduce shedding of HPAI H5N1 clade 2.2.1.2 in broiler chickens

HA.Hussein¹; Khedr, .M²; Suliman, RA²; Mohamed, MF²; El Safty, MD²

¹Department of Virology, Faculty of Veterinary Medicine, Cairo University.

²Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo.

Groups of one day old commercial broiler chicks were divided in to 8 groups 1, 2 and 3 were vaccinated with a prepared vaccine contain 500HAU of H5N1 reassortant antigen; while group 4, 5 and 6 were vaccinated with an imported reassortant vaccine with 500HAU antigen content of H5N1 at 1, 5 and 10 days of age; respectively. A group 7 was positive challenged control and group 8 negative challenged groups. Antibody titers were determined by HI test. All vaccinated groups were challenged 4 weeks post vaccination and tracheal and cloacal swabs were taken at 3, 5, 7, and 10 days post challenge and tested by real time RT-PCR (rRT-PCR) and virus isolation and titration in SPF ECE. Results of HI demonstrated that the groups vaccinated at 10 days of age were significantly higher compared to others with maximum titers at 4 weeks post vaccination. The protection % post challenge revealed 0, 20, 86 % and 0, 20 and 86 % in groups 1, 2, 3 and groups 4, 5, and 6; respectively. Results of rRT-PCR and virus isolation revealed that all chicken groups vaccinated at 1 and 5 days of age revealed 100% shedding at 3rd, 5th, 7th and 10th days post challenge. However, groups 3 and 6 which were vaccinated at 10 days of age demonstrated different shedding pattern where group 3 (vaccinated with local prepared 500HAU vaccine) showed at the 3rd and 5th days shedding by rRT-PCR and 80% and 20 % of the chickens in tracheal swabs and 80% and 40% in cloacal swabs when tested by virus isolation in eggs at 3 and 5 days post challenge; respectively.. Vaccination against H5N1 AIV is greatly affected by both antigen content of vaccine and level of maternal immunity in vaccinated chicks.

Keywords: Avian influenza; Antigen content; Maternal immunity; Challenge; rRT-PCR.



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Interfering of maternal derived antibodies with the protection of local inactivated reassortant H5N1 Avian influenza vaccines with antigenic content of 300HA units in commercial broiler chickens

Khedr, .M¹; Suliman, RA¹; Mohamed, MF; El Safty, MD¹ & HA.Hussein²

¹ Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo.

² Department of Virology, Faculty of Veterinary Medicine, Cairo University.

Eight groups of one day old commercial broiler chicks were kept in isolators along all the study. Groups 1, 2 and 3 were vaccinated with the prepared influenza virus vaccine ; group 4, 5 and 6 were vaccinated with one of the imported H5N1 reassortant containing 300 HAU at 1, 5 and 10 days of age; respectively. Groups 7 and 8 were positive and negative groups for the challenge trial. Blood samples were collected weekly for 4 weeks of age and tested by HI test. Post challenge, tracheal and cloacal swabs were collected at and tested by both real time RT-PCR (rRT-PCR) and virus titration in SPF eggs. HI test revealed no significant difference between groups in the first 3 weeks post vaccination and group 2 showed lower significant statistical difference. Results of the challenge trial revealed 0, 14, 80 % and 0, 14 and 86 % of protection in groups 1,2,3 and groups 4, 5, and 6; respectively. RT-PCR and virus isolation revealed that all chicken groups vaccinated at 1 and 5 days of age demonstrated 100% shedding at 3, 5, 7 and 10 days post challenge. However, groups 3 and 6 which were vaccinated at 10 days of age revealed difference in shedding pattern where group 3(vaccinated with local prepared vaccine) showed 100 shedding by rRT-PCR and 100%, 60% and 60 % of the chickens in tracheal swabs and 100%, 80% and 60%in cloacal swabs when tested by virus isolation in eggs at 3, 5 and 7 days post challenge; respectively. Indeed, there is evidence of interfering of maternal antibodies to vaccination at 1, and 5 days. Also, the 300HAU of antigen in the prepared avian influenza H5N1 vaccine are not enough in reduction of virus shedding post challenge.

Keywords: Highly pathogenic avian influenza; Immunity; Antigen content; Vaccination.



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Pathogenicity and genetic characterization of chicken anemia virus from Egypt

Sara Abdel- Mawgod¹, Amany Adel¹, Abdel Satar-Arafa¹ and Hussein A.Hussein²

¹Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, P.O. Box 264-Dokki, Giza- Egypt, 12618

²Virology.Dept, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt, 12211

Chicken Anemia Virus (CAV) is an important pathogen associated with immunosuppression in chicken. In this study, twelve samples were CAV positive out of 115 commercial poultry farms. The sequences of VP1 gene were aligned and compared to reference CAV strains as well as the phylogenetic analysis of these sequences. The results revealed that nine Egyptian CAV viruses were clustered with genotype II strains and the other three isolates were clustered with genotype I strains. The nine viruses had the highly pathogenic motif Q141 and Q144. Whereas the other three viruses had both amino acid motifs of low and high pathogenic strains (I75 and E144 of low and I125, Q141 and Q144 of high pathogenic strains). For further genetic analysis, three isolates (CAV/CA1, CAVGZ1 and CAV/SK4) were selected for full genome sequencing and the results revealed that the VP2 gene had two substitutions at V153 and E 175, while VP3 gene showed only one substitution at C118. To evaluate virus pathogenicity, 2 isolates were selected from each genotype (CAV/SK4 of genotype I and CAV/CA1 of genotype II). One-day-old specific pathogen free chicks were intramuscularly inoculated with each isolate in separate groups. After 18 days post infection, the 2 isolates showed positive percent 90% and 75% by PCR for CAV/CA1 and CAV/SK4 respectively. The **packed cell volume** values for them were 0.19 and 0.21 respectively. CAV/ CA1 and CAV/ SK4 isolates showed pathogenic evidences at the level of genetic and pathogenic studies with different degree of virulence as CAV/CA1 was more pathogenic than CAV/SK4.

Keywords: CAV; Molecular characterization; Full genome sequence; Pathogenicity evaluation; Chicken; Egypt.



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Enhanced protection and reduction in shedding in commercial broiler chickens vaccinated simultaneously with H5 inactivated and avian poxvirus vaccines and challenged with HPAIV H5N1 clade 2.2.1.2

Hussein.HA1*; El Safty. MM2; Mohamed. MF2; Suliman. RA2 and Zaki, AG3

1. Department of Virology, Faculty of Veterinary Medicine, Cairo University 12211.
- 2 Central Laboratory for quality control of Veterinary biologics, Abassia, Egypt
- 3 IFT corporation

In the present, chickens in two commercial broiler houses (15000 chicks each) were vaccinated with H5 inactivated vaccine, one of them was simultaneously vaccinated with avian poxvirus vaccine at 10 days of age. At 25 and 30 days of age, chickens from each house were transferred to the laboratory in isolators and challenged with the Egyptian H5N1 AIV clade 2.2.1.2 (A/Chicken/EG/1575s/2015). None vaccinated commercial broiler chickens which were included as positive and negative control groups. Protection % in vaccinated group with both Pox+H5 vaccines were 80% and 86.6% and in the H5 vaccinated group were 66.6% and 80% when challenged at 25 and 30 days of age. Testing of tracheal swabs collected from challenged chickens at 3, 5, 7 and 10 days post challenge at 25 days and 30 days of age by inoculation in SPF eggs revealed shedder % of 6.6; 13.3; 6.6 and 0 and % of 13.6, 6.6; 0 and 0 in the group vaccinated with Pox+H5 vaccines and % of 20; 33.3; 26.6 and 26.6 and 26.6; 33.3, 33.3 and 20 in the group vaccinated with H5 vaccine alone; respectively. Serology profile and histopathology of dead birds in all challenged groups were carried out. Indeed, the study reports the field effectiveness of H5 vaccine and the effect of simultaneous use poxvirus vaccine in broiler chickens.

Keywords: H5N1; Avian poxvirus; Prime boost; Shedding; Inactivated H5 vaccines.



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Amino acid substitutions in known antigenic sites and many human receptor signature in the HA gene of H9N2 AIVs isolated from commercial chickens in Egypt

El Sayed. RE1; Hussein. HA; Adel. AI3 and Bazid. AI4

1.Esmailia Misr Arab poultry company. 2 Departemnt of Virology, Fac. Vet. Med, Cairo University. 3 RLQP, AHRI-Dokki, Egypt. 4 Department of Virology, Fac. Vet. Med, Sadate city University, Egypt

In the present study, 51 tracheal swabs were collected in the period between 2014 to 2016 from broiler flocks demonstrating mortalities ranged from 20 to 60%. Testing of these samples by real time RT-PCR for H9 revealed 66% were positive. Ten viruses were isolated and the complete HA gene was sequenced. Phylogenetic analysis of the sequences revealed clustering of the isolated viruses with other Egyptian H9N2 strains isolated in 2011 and fall in the Asian G1-like lineage. Amino acid identity percent between the isolated viruses and the prototype A/Quail/ Hong Kong/G1/97 H9N2 virus ranged between 86.5 to 89.9%. On the other hand, Amino acid identity between the isolated viruses ranged from 91.2% to 99.8%. Striking amino acid substitutions in antigenic sites with loss of some glycosylation sites were exist. Many human receptor signatures were also found in the HA sequences of the isolated H9N2 viruses. Lack of multiple basic residues in the cleavage site indicated the low pathogenicity of the isolated viruses. The findings in the present study indicated the rapid and continuous evolution of the Egyptian H9N2 virus circulating in chickens which may lead to evolving new antigenic variants.

Keywords: AIV H9N2; Mutations in HA gene of influenza viruses; G1-like AIV.



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Enhancing pathogenicity of low-pathogenic H9N2 avian influenza virus after vaccination with live attenuated infectious bronchitis vaccine

Zainab, M Ismail1; El Deeb, AH1; El Safty, MM2 and HA. Hussein1

1Department of Virology, Faculty of Veterinary Medicine, Cairo University, Giza-Egypt12211.

2 Central Laboratory for Evaluation of Veterinary Biologics, Abassia-Egypt

In the present study, two experiments were carried out for studying the pathogenicity of H9N2 AIV in broiler chickens after vaccination with different live respiratory viral vaccines. One-day- old specific pathogen free (SPF) chicks were divided into four groups in each experiment. In experiment 1; groups 1 and 2 were inoculated with H9N2 AIV via nasal route at 1st day old, groups 1 and 3 were vaccinated with live IBV vaccine at 5th days old and group 4 was left as a negative control. In experiment 2; groups 5 and 6 were inoculated with AIV subtype H9N2 via nasal route at 1st day old, group 5 was vaccinated with live IBV vaccine and live NDV vaccine at 5th days and 18th days old; respectively, groups 6 and 7 were vaccinated with live NDV vaccine at 18th days old and group 8 was left as a negative control. Tracheal and Cloacal swabs were collected at 3rd, 5th, 7th, 10th, 12th and 15th days old from all groups in experiment 1; 3rd, 5th, 7th and 10th days old from all groups in experiment 2. Quantitative Real time (rRT-PCR) was applied on the collected tracheal swabs for detecting RNA copies of H9N2 AIV and revealed a significant increase in H9N2 AIV titer with extension in the period of viral shedding in groups 1 and 5. Cloacal swabs and the positive rRT-PCR tracheal swabs were inoculated in 10- day- old SPF embryonated chicken eggs (ECE) to confirm rRT-PCR results. Histopathological examination of the collected internal organs revealed severe lesions in groups 1 and 5. Weekly collected sera for detecting antibodies against H9N2 AIV, NDV and IBV from all chicken groups revealed a significant increase in the titer. In conclusion results demonstrated the increase in pathogenicity of H9N2 AIV.

Keywords: H9N2 avian influenza virus; Shedding; Enhanced protection; IBV vaccine.



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Organized by Faculty of Veterinary Medicine, Cairo University, Egypt
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Host range and pathogenicity determinants of the reassortant H9N2 viruses circulating in backyard chicken in Egypt

Abdelhafez Samir¹, Amany Adel¹, Abdelsatar Arafa¹, Hesham Sultan² and Hussein A. Hussein^{*3}

¹Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, P.O. Box 264-Dokki, Giza- Egypt, 12618. ²Avian and Rabbit Diseases Dept., Faculty of Veterinary Medicine, University of Sadat, City Sadat, Minoufiya, Egypt
³Virology Dept., Faculty of Veterinary Medicine, Cairo University, Giza, Egypt, 12211

Reassortment is a genetic mechanism for evolution of influenza viruses and it was recorded extensively in H9N2 viruses. Since the introduction of H9N2 virus in Egypt in 2011, continuous evolution including different mutations in isolated strains from commercial and backyard chickens were reported in previous studies. In the present study, we isolated five reassortant H9N2 viruses from backyard chickens. Sequence analysis of the 5 genes (HA, PB2, PB1, PA and NS HA) revealed that hemagglutinin gene is genetically related to the G1 lineage in group B, similar to the circulating H9N2 strains in Egypt since 2011 however sequence of the other four genes revealed that these viruses are reassortant viruses and genetically related to group A of G1-like viruses. The pathogenic determinant genes showed reassortment event with different Eurasian subtypes that have genetic signatures for the avian-mammalian transmission; particularly the non-structural gene was genetically inherited from the highly pathogenic H7N7 which was reported in human cases contact to infected chicken population. Amino acid residues characteristic of the highly pathogenic strains were determined (S42, V127, L550, L672 and V504 in the internal genes NS1, PA and PB2; respectively). Indeed, the study reports the circulation of reassortant H9N2 viruses with distinct molecular determinants which could enable these viruses to transmit and adapt from avian to mammalian.

Keywords: H9N2 AIV; reassortment; (HA, PB2, PB1, PA and NS HA); AIV evolution.



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Assessment of some mycobacterial antigens in bovine tuberculosis diagnosis

Ahmed Orabi¹ Kamelia Osman¹, Emad .M. Riad², Wagdy Samir²

¹ Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt. ² Tuberculosis unit, Animal Health Research Institute, Cairo, Egypt.

In this study the bacteriological examination of 39 tuberculin positive animals from Egyptian farms by cultural methods revealed that 29 recovered on Lowenstein- Jensen and middle brook media with a percentage reached 74.36%. Microscopic examination was conducted for all 39 tuberculosis lesions by using Ziehl-Neelsen stain and 27 were positive with a percentage of 69.23%. Real time PCR was carried out on all 29 isolates for identification of recovered colonies and all were identified as *Mycobacterium* species with a percentage of 100%. Some *M. bovis* cell extract antigens “sonicated antigen (SA)”, culture filtrate antigens "short term culture filtrate (ST-CF)" and tuberculin were prepared. Extraction of *M. bovis* with sonication resulted in the recovery of 1.3 mg/ml of soluble protein in the sonicated extract and the protein concentration of *M. bovis* ST-CF at 2nd week of growth was 2.7 mg/ ml. the protein concentration of *M. bovis* prepared tuberculin was 2.0 mg/ml and 1.0 mg/ml of offered PPD. SDS-PAGE was applied for characterization of antigenic extracts and CF of *M. bovis* and Western blotting analysis was performed. The use of ELISA on serum samples collected from the 1540 cows, before being tested by SICTT, revealed that 131 (8.51%) animals were positive to bTB by using PPD, 139 (9.03%) by using the commercial mixture antigen, 120(7.79%) by using SA, 125(8.12%) by using ST-CF and 133(8.64%) positive animals by using prepared tuberculin, indicating that the use of mixture antigen could help to give more effective diagnosis. This study proved that the superiority of ST-CF, prepared tuberculin and bovine PPD over SA extracts using ELISA in distinguishing between infected and non-infected cattle.

Keywords: Bovine Tuberculosis; ELISA; Antigens; Tuberculin test.



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Serological and molecular detection of subclinical paratuberculosis infection in cattle of dairy herds in Egypt

Nasr, E.A; Abdelrahman, M.; Shereen, A.M.; Marwah , M. Mohamed; Amr Zeyada

Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt.

Johne's disease (JD) is a chronic enteric disease in ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Disease progression follows four distinct stages; silent, sub clinical, clinical and advanced. In this study, the presence of paratuberculosis was investigated by serological and molecular methods in different herds of dairy cattle. Blood, milk and stool samples of 155 cows (out of 2500) aged 3 years old or older with chronic diarrhea were collected. Indirect paratuberculosis enzyme – linked Immune sorbent assay (ELISA) was used for serological investigation, Polymerase Chain Reaction (PCR) was utilized for molecular identification of MAP from milk and stool samples according to ELISA, 18(11.6%) serum samples were positive in PCR of milk and stool samples, MAP DNA was detected in 22 (14.2%) and 42 (27.1%) of samples, respectively. In this study, paratuberculosis was found at high rates in dairy cattle in Egypt. In conclusion, it was detected that symptoms in paratuberculosis were subclinical and not always observed and the use of diagnostic laboratory method may be an important aid in revealing diseases in dairy farm.

Keywords: Paratuberculosis; Dairy cattle; ELISA; PCR.



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Molecular characterization of virulence genes in *Campylobacter* species in chicken in Egypt

**Hamouda, A. G.¹; Mona, M. Sobhy²; Marouf, S.³; Ghanem, H.E.A.⁴; Bastamy, M. A.¹;
Nagwa, S.Rabie⁵ and Kotb, M.H.R.²**

¹: Dept. of Poultry and Poultry diseases, Fac of Vet Med. Cairo Univ. Giza, Egypt. ²: Dept. of Reproductive diseases, ARRI, ARC, and Giza, Egypt ³: Dept. of Microbiology, Fac. of Vet. Med. Cairo Univ. Giza, Egypt. ⁴: Major Vet in Egyptian Army, Veterinary Sector. Nasr City, Egypt. ⁵: Dept. of Poultry diseases, National Research Center, Dokki, Giza, Egypt.

This work was done to investigate the molecular characterization of *Campylobacter jejuni* and *Campylobacter coli* isolated from cloacal swabs from chicken, intestinal content, gizzard, liver, chicken eggs, water and ration from chicken farms. A total of 498 chicken samples were examined by the conventional methods for isolation and identification of *Campylobacter* species. Then isolates were subjected to a standard phenotypic identification of *C. jejuni* and *C. coli* by PCR using specific primers for hippuricase gene. The prevalence of *Campylobacter* isolates was 21.89%; from cloacal swabs 23.77%, intestine 24 %, liver 22 %, 21.48% from chicken eggs, 12% water and 6.67% from ration. Out of 109 identified isolates, 81(74, 3%) were *C. jejuni* while 28(25.7%) were *C. coli*. A multiplex-PCR method was developed for the detection of *C. jejuni* and *C. coli* using specific primers. The virulence genes of *C. jejuni* (*FlaA*, *virB11*) have been shown at 855 bp and 494 bp respectively. Whereas the cytolethal distending toxin (*cdtB* and *cdtC*) of *C. jejuni* have been shown at 495 bp and 555 bp respectively. The results of the present study showed that all analyzed isolates of *C. jejuni* and *C. coli* contained the *flaA* gene. On the other hand, the *virB11* gene was present only in 10 of 81 of the analyzed isolates, whereas most of the strains 32 (39.51%) contained *Cdt* genes. Finally, we concluded that PCR detection and discrimination of *Campylobacter* virulent can be utilized as a simple and rapid tool to therapeutic and preventive strategies against *Campylobacter* infection in chicken are insisting recommended.

Keywords: *Campylobacter* species; Virulence genes; Chicken; Egypt.



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First report of *Bordetella avium* in Egyptian turkey flocks

Erfan, A.M.¹, Jihan M. Badr² and Mahmoud H. Abd-elhalim³

¹Reference Laboratory for Veterinary Quality Control on Poultry Production,

²Poultry Diseases Department, Animal Health Research Institute, Dokki- Giza -Egypt

³Brucella department, Animal Health Research Institute, Dokki- Giza -Egypt

To screen *Bordetella avium* in Egyptian turkey flocks, tracheal swabs and nasal exudates were collected from 21 turkey farms from 5 Egyptian governorates. Bacteriological examination revealed the isolation of 2 strains. When antibiogram was performed, the 2 isolates showed variable resistance to 10 tested antibiotics. Both strains produced guinea pig hemagglutination and were able to adhere to the tracheal rings. Confirmatory *recA* gene PCR was performed. On the other hand, *blaTEM*, *tetA(A)*, *aadA1*, *sul1* and *dfrA* genes were amplified in both *B. avium* strains. Partial sequencing of the amplified 740 bp of *recA* gene revealed 100% maximum identity with both *B. avium* German strain ATCC 35086 and American strain 197N. To the best of our knowledge, this is the 1st record of *B. avium* in Egyptian turkey flocks.

Keywords: *Bordetella avium*; Turkey flocks; Egypt.



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Preparation of necrotic enteritis vaccine for turkey

Elham F. El-Sergany; El-Helw, H. A.; Hala El-Sawy; Taha, M. M.; Abdalla, Y. A., El-Meneisy, A. A.

Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt

Clostridium perfringens is the most important cause of enteritis in domestic animals, in chicken and turkey it well known as pathogen responsible for necrotic enteritis; hepatitis, and cholecystitis. The disease in turkey characterize by either severe form with high rate of mortalities or subclinical form of reduce growth rate and increase condemnation rate. The major factor responsible for pathogenicity of *C. perfringens* is alpha toxin. In this study immunization of turkey with *C. perfringens* type A toxoid vaccine with different doses depend on lethality of toxin (24; 48, and 96 Minimum Lethal Dose). Antibody titer elicited by vaccination was measured by toxin neutralization test; ELISA, and challenge test. It revealed that antibody titer expressed by international antitoxin unit per ml was 7.4; 4.1, and 1.26 respectively according to above mentioned dose, and also the protection % against challenge was 100% when vaccinated with either 48 or 96 MLD, while it gave 80% when vaccinated with 24 MLD. Also these finding demonstrated that the vaccine able to protect turkey for 6 months. It concluded that use of *C. perfringens* alpha Toxoid with recommended dose of 48 MLD able to protect turkey for 6 months.

Key words: *Clostridium perfringens* type A; Alpha toxin; Turkey; Vaccine.



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Preparation of Buffered Acidified Plate Antigen from *Brucella abortus* strain 19

L. F .Farahat¹, K. A. Abd El-Azeem¹, A. El-Menisy¹, A.Mahrouse², E. A. Nasr¹.

¹Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt.

²Department of Public Health and Zoonoses, General Organization for Veterinary Services.

As one of the major zoonotic infections worldwide, brucellosis continues to be a nagging public health problem that results in significant morbidity and economic losses. Accurate diagnosis must include laboratory tests that allow the direct *Brucella* isolation or indirect detection of antibodies. Being practical, serological tests are routinely used for the diagnosis of brucellosis. The rapid acidified agglutination tests, viz. the buffered acidified plate antigen (BAPA) and the brucellosis card (BCT) tests, deactivate IgM mostly responsible for nonspecific reactions. The BAPA, final pH 4.0, was developed for testing bovine and porcine sera to reduce the number of nonspecific plate agglutination test reactions and to decrease testing costs. The test is slightly more sensitive than the card test. This American antigen has been used in a presumptive test for brucellosis in Egypt since 1987. Antigen for the BAPA is prepared from *B. abortus* biovar 1 strain S1119-3 according to the USDA SOPs. The current study validates the preparation of BAPA antigen from the vaccinal *Brucella abortus* biovar 1 strain 19 as an alternative to the USDA strain S1119-3. For this purpose, a total of 4100 bovine sera from five farms located in different governorates were screened for brucellosis. The effectiveness of this BAPA prepared from strain 19 was compared with the standard BAPA and BCT antigens prepared from *B. abortus* strain 99. Evaluation was done by using a panel of known dilutions of the OIEISS as the international reference standard serum. There were no significant differences in results of the BAPA antigen prepared from strain 19 and the conventional antigen prepared from strain 99. The authors concluded that the vaccinal strain 19 can be used in lieu of strain 99 to prepare the BAPA antigen.

Keywords: *B. abortus*; Buffered acidified plate antigen; Rapid acidified agglutination tests.



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Using of multiplex PCR for detection of some microbial pathogen causing pneumonia in camel

Amani A. Hafez

Doctor of Bacteriology, Infectious Diseases Unit, Animal Health Department, Animal Production and Poultry Division, Desert Research Center, 1-Mathaf El-Mataria St. 11753, El-Naam, Cairo, Egypt

Pneumonia is one of the most important causes of morbidity and mortality, which causes significant economic losses in camels (*Camelus dromedarius*). This study was designed to compare the fast-responsive PCR with conventional culture method in pneumonia and evaluate the reliability of multiplex PCR assay (mPCR) for simultaneously detecting and identifying six pathogenic bacteria. So, 160 nasopharyngeal swabs were collected from 160 camels from Matrouh and Alexandria Governorates, Egypt, suffering from pneumonia. The result revealed that, (36.8%) and (51.2%) isolates were obtained by culture and PCR respectively. The isolates constituted 6 genera of pathogenic bacteria, were as follows, *Klebsiella* spp., (12.5%) and (16.2%), *E. coli*, (10.6%) and (13.1%), *Staphylococcus* spp., (9.4%) and (12.5%), *Salmonella* spp., (2.5%) and (4.3%) and *Pasteurella multocida* (1.9%) and (3.1%) by culture and PCR respectively and to the total number of isolates. Meanwhile, *Mycoplasma* spp., (1.9%) were detected only by PCR. Multiplex PCR assay was found to be rapid, economic and sensitive tool for accurate detection of the six pathogenic organisms concurrently. In addition, mPCR detected virulence genes of these pathogens enhancing evaluation of the pathogenicity of pathogenic bacteria present in infected camels and is a useful and rapid technique to apply to field samples.

Keywords: *Camelus dromedarius*; Pneumonia; Culture; mPCR.



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Molecular studies on some virulence factors of *Pseudomonas aeruginosa* isolated from chickens as a biofilm forming bacteria

Maram, M. Tawakol¹, Nehal, M. Nabil¹ and Reem, M. Reda²

¹ Reference Laboratory for Veterinary Quality Control on Poultry production-Dakahlia branch-(Gamasa).

² Reference Laboratory for Veterinary Quality Control on Poultry production-Dokki lab. - Giza.

This study was aimed to isolate and identify *Pseudomonas aeruginosa* (*P. aeruginosa*) from 150 diseased broiler chickens in addition to 50 environmental swabs from water pipes and tanks to examine their susceptibility against some common usable antimicrobial agents in addition to detection of some virulence genes using Polymerase Chain Reaction technique (PCR) and evaluation of its ability to form biofilm in vitro. Clinically the affected chickens were subjected to postmortem examination (P.M) then samples from internal organs such as (liver, heart, lung, spleen, and intestine); tracheal and environmental swabs were collected and subjected to bacteriological examination and identification. Twenty *P. aeruginosa* isolates were recorded from the diseased chickens and environmental swabs with an incidence of (10%). Sixteen isolates were recorded from internal organs of 150 diseased chickens with an incidence of (10.66%); meanwhile 4 isolates were recorded from 50 water pipes and tanks swabs with a percentage of (8%). Antibioqram sensitivity testing showed highly sensitivity to amikacin, ciprofloxacin, colistin sulphate and norfloxacin with percentages of (90%), (90%), (90%) and (70%) respectively. Doxycycline and penicillin showed resistance with a percentages of (75%) and (65%) respectively. PCR technique was a good tool for testing three virulence genes; *pslA*, *pelA* and *fliC* genes; the three genes were detected in of the examined samples with a percentage of (100%). A significant relationship between the existence of three virulence genes in the isolated *P. aeruginosa* and ability of biofilm formation (Slime producing ability) was reported in this study.

Key words: *Pseudomonas aeruginosa*; Virulence genes; Biofilm; Chicken.



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Investigation of contagious bovine pleuropneumonia vaccination in Khartoum State, Sudan 2016-2017

**Eslah, A.D.O.¹, Neimat, M.E.E¹, Hamid.F.M.A¹, Mohammed.G.E², Ibrahim,
M.T.³**

¹ Animal Resources Research Corporation, Central Veterinary Research

Laboratory.² College of Veterinary Medicine Sudan University Of Science &Technology.

³ College of Animal Production Science & Technology, Sudan University of Science & Technology

This study was aimed to determine the impact of CBPP vaccination as a risk factor in the prevalence of CBPP in Khartoum state. Cross-sectional study was conducted from November 2016 to May 2017 in various pastoral areas in Khartoum state assessed firstly using well designed questionnaire from the animal owners and the pastoralists. A total of 386 sera were examined for the incidence of specific antibodies against *Mycoplasma mycoides* subsp. *mycoides* small colony (*MmmSC*), using a competitive enzyme-linked immunosorbent assay (cELISA). The results showed that from 300 non-vaccinated samples 46% were positive and 54% were negative while the 86 vaccinated sample 43% were positive and 57% were negative result. This obliges the implementation of appropriate vaccination program and control measures to reduce the economic losses associated with CBPP.

Keywords: Contagious Bovine Pleuropneumonia; Vaccination; Sudan.



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Molecular characterization of *Riemerella anatipestifer* isolated from ducks' outbreaks in Egypt

Heba Naim

Department of Microbiology, Faculty of veterinary Medicine, Cairo University

Riemerella anatipestifer (*R. anatipestifer*) is one of the most important threats to duck rising, particularly in developing countries, where they depend on duck as a source of protein in their diets. The aim of this study was microbiological isolation of *R. anatipestifer* from Egypt farms (30 birds; ducks and ducklings), molecular characterization, genetic analysis of the isolates based on full length outer membrane protein (OmpA), sequencing, BLAST analysis, and phylogeny based on full length *ompA* gene were conducted, the results were confirmed by 3D prediction of protein.

Five *R. anatipestifer* were isolated from the examined birds. Results showed invasion of a few different genotypes among the Egyptian duck flocks, indicating progressive circulation of *R. anatipestifer* among these flocks. Phylogeny classified the diversity of *R. anatipestifer* worldwide into two main lineages; each lineage diversified into three main clusters. Our study reports the first genotyping of *R. anatipestifer* based on an immunogenic protein (OmpA) and confirm the infiltration of different *R. anatipestifer* clusters into the Egyptian duck flocks. Our isolates were found to belong to the two lineages.

These findings are starting points for advanced investigations of the genetic diversity of *R. anatipestifer* at national and regional levels to better understand their genetic relatedness. Although until now, at least 21 *R. anatipestifer* serotypes have been identified, the molecular techniques regarding its diagnosis, pathogenicity, and antigenicity are not well-attained. So, deeper studies should be done to stop this catastrophic loss of such a valuable protein source.

Keywords: Ducks; *R. anatipestifer*; OmpA; 3D.



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Silver nanoparticles and their antibacterial activity, histopathological effects in rainbow trout (*Oncorhynchus mykiss*)

Mohamed Shaalan¹, Magdy El-Mahdy¹, Mansour El-Matbouli², Mona Saleh²

¹Department of Pathology, Faculty of Veterinary Medicine, Cairo University, 12211, Giza, Egypt. ² Clinical Division of Fish Medicine, University of Veterinary Medicine, Veterinärplatz 1, 1210, Vienna, Austria.

Microbial antibiotic resistance is a major concern in both human and veterinary medicine. The excessive use of antibiotics is one of the most important causes for this problem. Silver nanoparticles have been proven to show bactericidal effects against different fish pathogens *in vitro*. The aim of this study was to evaluate the antibacterial effects of silver nanoparticles *in vivo* against *Aeromonas salmonicida*, the causative agent of fish furunculosis. Rainbow trout (*Oncorhynchus mykiss*) (n = 90) were divided into three groups; 30 fish challenged with *A. salmonicida*, 30 fish challenged with *A. salmonicida* then immersed in silver nanoparticles, and 30 fish as a negative control group. Upon completion of the experiment, fish were euthanized, and then kidney and spleen were sampled for bacteriological investigation, DNA extraction and histopathology. The onset of clinical signs started three days after the bacterial challenge in the infected group only as multiple furuncles. *A. salmonicida* was re-isolated from skin lesions and PCR results confirmed the presence of *A. salmonicida* in the infected group only. Histopathology of the infected group revealed the presence of bacterial aggregates in the fish tissues with infiltration of inflammatory cells. Silver nanoparticles-treated and negative control groups didn't show any clinical signs, mortalities or histopathological alterations and they were tested negative for *A. salmonicida* with PCR. Based on our results, we concluded that silver nanoparticles are effective against *A. salmonicida* infection. They could be adopted for the development of potential antibacterial agents to reduce dependence on antibiotics in aquaculture.

Keywords: Silver nanoparticles; Fish diseases; Nanomedicine; Histopathology.



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Regulation of muscle mass growth via microinjection of CRISPR/Cas9 protein into channel catfish, *Ictalurus punctatus*, one-cell embryos targeting myostatin gene.

Karim Khalil^{1,2} and **Rex Dunham**²

¹ Anatomy and Embryology Department, Faculty of Veterinary Medicine, Cairo University.² School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University.

The myostatin (MSTN) gene is important because of its role in regulation of skeletal muscle growth in all vertebrates. Gene knockout has been used to study these gene functions in vivo. The clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9 (CRISPR/Cas9) system is a powerful tool used to edit genomic DNA sequences to alter gene function. While the traditional approach has been to introduce CRISPR/Cas9 mRNA into the single cell embryos through microinjection, this can be a slow and inefficient process in catfish. In this study, a detailed protocol for microinjection of channel catfish embryos with CRISPR/Cas9 protein is described. Briefly, eggs and sperm were collected and then artificial fertilization performed. Fertilized eggs were transferred to a Petri dish containing Holtfreter's solution. Injection volume was calibrated and then mixture of guide RNAs and Cas9 protein targeting the channel catfish, *Ictalurus punctatus*, muscle suppressor gene MSTN were microinjected into the yolk of one-cell embryos. CRISPR/Cas9 induced high rates (88–100%) of mutagenesis in the target protein-encoding sites of MSTN. MSTN-edited fry had more muscle cells ($p < 0.001$) than controls, and the mean body weight of gene-edited fry increased by 29.7%. The predicted protein sequence alterations due to these mutations included frame shift and truncated protein due to premature stop codons. We aim to produce growth-enhanced lines of channel catfish with CRISPR technology. These gene-edited lines would then be fully characterized for growth rate, feed efficiency and disease resistance and compared to genotypes currently used in aquaculture.

Keywords: Myostatin gene; CRISPR/Cas9 protein; Catfish.



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Pharmacokinetics/Pharmacodynamics of intramammary cefquinome in lactating goats with and without experimentally-induced *Staphylococcus aureus* mastitis

S. A. El Badawy^{1,3} A. M. M. Amer¹ G. M. Kamel¹ K. M. Eldeib² & P. D. Constable³

¹Pharmacology Department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt; ²National Organization for Drug Control and Research, Egypt; ³Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA.

Values for pharmacokinetic variables are usually obtained in healthy animals, whereas drugs are frequently administered to diseased animals. This study investigated cefquinome pharmacokinetics in healthy goats and goats with experimentally induced mastitis. Five adult lactating goats received 75 mg of cefquinome intramammary infusion using a commercially available product into one udder half before and after induction of clinical mastitis. Clinical mastitis was induced by intracisternal infusion of 100 cfu of *Staphylococcus aureus* ATCC 29213 suspended in 5 ml of sterile cultural broth. Cefquinome concentrations were determined in plasma and skimmed milk samples using microbiological assay and high performance liquid chromatography. Pharmacodynamics was investigated using the California Mastitis Test, electrical conductivity, the sodium and potassium concentrations and pH of milk. Both MA and HPLC analytical methodologies yielded statistically similar mean values for the cefquinome concentration-time relationship in skimmed milk and plasma and similar values for almost all calculated pharmacokinetic indices; however, experimentally induced mastitis decreased the maximal cefquinome concentration and shortened the elimination half-time in milk when compared to healthy goats. In conclusion, mastitis facilitated the absorption of cefquinome from the mammary gland of lactating goats and induced marked changes in milk pH, sodium and potassium concentrations, emphasizing the importance of performing pharmacokinetic studies of antimicrobial agents in infected animals.

Keywords: Pharmacokinetics; Pharmacodynamics; Cefquinome; *Staphylococcus aureus* mastitis.



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Bactericidal effect of silver nanoparticles on Methicillin Resistant *Staphylococcus aureus* (MRSA) isolated from bulk milk tanks

Zakaria, I. M¹ and Walaa M. A. Elsherif²

¹ Animal Health Research Institute, Bacteriology Department, Dokki- Giza, Egypt

² Animal Health Research Institute, food Hygiene, Assiut Regional Lab., Egypt.

Recently, silver nanoparticles (AgNPs) have been widely used in various applications as antimicrobial agents, anticancer, diagnostics, biomarkers, cell labels, and drug delivery systems for the treatment of various diseases. The present study investigated the bactericidal effect of AgNPs against methicillin resistant *S. aureus* (MRSA) isolated from bulk milk tanks. A total of 105 bulk milk tank samples were collected from cows, buffaloes and mixed milk of both (35 milk samples of each). The mean total staphylococcal count was $4.2 \times 10^3 \pm 6.1 \times 10^2$, $3.8 \times 10^3 \pm 5.9 \times 10^2$ and $1.1 \times 10^4 \pm 7.6 \times 10^3$. MRSA strains were identified in 8, 4 and 11 isolates out of 10, 11 and 15 positive *S. aureus* strains isolated from cows, buffaloes and mixed milk with incidence of 22.9%, 11.4% and 31.4% out of all samples respectively. MRSA isolates were only sensitive to vancomycin at percentage of 82.6% while, the resistance pattern of MRSA isolates revealed that they resist to most antibiotics used (multidrug resistant strains (MDR)). AgNPs solution was prepared, identified by Transmission Electron Microscopy (TEM) in nano size ranged from 18.1 nm: 29.3 nm and examined for bactericidal activity against MRSA by using well diffusion assay. The results showed the mean values of inhibition zone of 30, 60 and 90 μ l concentrations of Ag-NPs were 18.1 ± 0.31 , 21.3 ± 0.27 and 23.3 ± 0.29 , respectively. Also, the statistical analysis showed highly significant differences in bactericidal effect of different concentrations of Ag-NPs on MRSA strains.

Keywords: Silver nanoparticles; *S. aureus*; MRSA; Milk.



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The use of ultra sonication for preparation of nano-adjuvant rabies inactivated vaccine

Amany M. El-zieny¹, A.A.Farghali², S. I.El-Dek², A. F. Soliman¹, and Mohamed Samir³

¹Department of Rabies, Veterinary Serum and Vaccine Research Institute.

² Materials Science and Nanotechnology Department, Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Beni-Suef, Egypt.

³Department of Zoonoses, Faculty of Veterinary Medicine, Zagazig University

The usage of engineered nanomaterials that possess unique physicochemical properties in adjuvants enables researchers to potentially achieve improved protection against infectious diseases. Vaccination is a biological process that administers antigenic materials to stimulate an animal's immune system to develop immunity to a specific pathogen. Rabies is a preventable disease, and as a major control strategy, vaccination is recommended by the World Health Organization (WHO). The vaccine is prepared by inactivation of rabies virus to prevent disease either before or after exposure to the virus. Recently, several studies were conducted on the use of nanoparticles as adjuvant to enhance the immunogenicity of antigen which decreases the dose of immunologic adjuvants that are now being engineered to alter the natural adaptive immune response to an antigen for increased potency and duration of immunity.

Keywords: Nanoparticles as adjuvant; Inactivated Rabies Vaccine; Immunogenicity of antigens.



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Efficacy of Quercetin nanoparticles as a new antiviral against H5N1 influenza virus replication

Hemat S. El-Sayed¹, Wesam H. Mady², Mohamed A. Saif², Wesam A. Tawfik³

¹Department of Poultry diseases, Animal Health Research Institute, Benha Branch, Benha, Egypt
Laboratory for Veterinary Quality Control on Poultry Production, Animal Reference²Health
Research Institute, Dokki, Giza, Egypt

³Naqaa foundation for scientific research, Technology and Development, Giza, Egypt

The growing emergence of H5N1 Avian influenza virus (AIV) mutants in last years calls the urgent need for new effective antiviral drugs against AIV. Quercetin, a promising natural candidate for treatment and prevention of AIV infection but unfortunately, low solubility and poor bioavailability of quercetin hindering its use in the medical field. Nanotechnology offer innovative solution for enhancement of quercetin solubility and bioavailability and subsequently boosts its therapeutic effect. In the current study quercetin nanoparticles were prepared by nanoprecipitation technique, quercetin nanoparticles showed a particle size range from 182 nm to 240nm at flow rate 10 ml/min, S/AS volume ratio 1:12, stirring speed 1300 rpm and drug concentration 5mg/ml. The determined safe dilution of quercetin nanoparticles 10µg/ml had a significant inhibitory effect on the avian influenza virus H5N1 strain (EPI573317) indicated by reduction the virus titer and the CPE in MDCK cells. Quercetin nanoparticles could be used as a new promising inhibitor for H5N1 AIV and expected to be on the top of candidates as antiviral against influenza virus in the near future.

Keywords: Antiviral; Quercetin nanoparticles; H5N1; Influenza virus; nanoprecipitation technique; Nanotechnology.



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Developmental interventions for biorisk management in livestock sector- the dairy science park approach

Muhammad Azam Kakar

Faculty of Biotechnology and Informatics, Balochistan University of Information Technology, Engineering and Management Sciences Takatu Campus, Airport road Quetta, Pakistan

Dairy Science Park has emerged for productive utilization of the available livestock and poultry resources in Pakistan and the adjoining regions of Afghanistan, Tajikistan and China, focusing at self-employment for the youth and hygienic food production for the local and international Halal market. Entrepreneurship development for the outgoing university graduates and biorisk management is an integral component of this chain of activities, to identify and eradicate various entry points for health hazards into the food value chain. An international consultation has been in progress since several years and consultative meetings and workshops were held at Amsterdam, Dubai, Bangkok, Pukit and Colombo to integrate the biorisk management concepts into the university curriculum and make aware the policy makers, farmers, business community and the academicians about seriousness of this issue and its integration into their respective fields.

The Livestock sector of Pakistan comprises is supported by a network of veterinary hospitals and artificial insemination centers under the livestock and dairy development department, extension wing, spread up to village council levels. In addition the research wing of the department takes care of disease diagnosis and vaccine production. DSP Innovations has been established as a private firm to bring innovations in the existing farming system in the private sector. Dairy Science Park suggests registration and certification of the livestock/poultry production, processing and marketing facilities and service providers for quality standards. All these interventions in Pakistan will definitely bring a positive change in the developmental interventions for Biorisk Management in Livestock Sector using the DSP approach.

Keywords: Biorisk; Management; Entrepreneurship; One Health; Livestock; DSP; Pakistan.



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Food hazards

“Permissible limits in Food”

Heba Naim

Department of Microbiology, Faculty of veterinary Medicine, Cairo University

The greater challenge is created by the development in the field of importing and exporting of food and food products which mean that any mistake can kill people not just locally but also globally. Microbe-contaminated food and water kill up to two million children in developing countries each year. There are many reported cases for hazards related to food. Even these percentages are not accurate, because food borne diseases are underestimated due to underreporting and difficulty to establish relationships between food and resulting illness or death. The concept of depending on laboratories only for assuring food safety is not completely right, it is just a helping tool especially in food industry. Permissible limits in Food is the food hazard (affect the food quality) added by our hands and causes sever health hazards to animals as well as human. Food safety is multi-sectorial and multidisciplinary and it is a shared responsibility between governments, food safety organizations (new food regulations), industry, producers, Universities and media (awareness campaigns) and consumers. A global cooperation between the organizations (responsible for food safety, human health, animal health), research centers all over the world, universities through an internal great project for evaluation and calculating the permissible limits per meal not per item in one type of food.

Keywords: Food safety; Permissible limits; Health hazards.



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The therapeutic impact of consuming camel milk and/or curcuma longa ethanolic extract on induced hepatocarcinogenesis in rats

Marwa M. Salah Khattab, Kawkab A. Ahmed and Hala M. F. El Miniawy

Department of Pathology, Faculty of Veterinary Medicine, Cairo University, El Giza square, Giza, Egypt.

Camel milk (CM) from free ranging camels and *curcuma longa* ethanolic extract (CLE) were said to exert a hepatoprotective effect. Therefore, this study was performed to investigate the therapeutic effect of CM and CLE on induced hepatocarcinogenesis in rats. Rats were divided into 8 groups (7 rats each). Group I was a control group. Group II was administered CM starting from the 28th week of the experiment. Group III was administered CLE extract (250mg/kg BW). Group IV was administered CM (5mL) and CLE extract (250 mg/kg BW). Group V, VI, VII and VIII were injected intraperitoneally by single dose of diethylnitrosamine (200 mg/kg BW) at day of the experiment and then received phenobarbitone (0.05%) in drinking water till 28th week. Group VI, VII and VIII were treated with CM, CLE extract and CM and CLE extract respectively. In the groups treated with CM and/or CLE extract, treatment started from the 28th week and ended at the 38th week. Liver specimen were collected for histopathological and immunohistochemical examination. The mean area of hepatocellular altered foci in H & E stained section and of placental glutathione-S-transferase (P-GST) positive foci were measured using image analyzer. Group VIII had the least mean area of hepatocellular altered foci and the least P-GST positive foci followed by group VI and then group VII. Camel milk had a good inhibitory effect on induced hepatocarcinogenesis in rats that was potentiated with CLE as evidenced by the reduced mean area of enzyme altered foci.

Keywords: histopathology; Immunohistochemistry; Placental gluathathion-S-transferase; Hepatocellular altered foci.



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Dietary conjugated linoleic acid modifies meat composition and fatty acid profile in growing rabbit

A.M. Abdelatty¹, H.O. Abu-Baker², SH. A. El-Medany³, M.A. Elhady⁴, O.A.Ahmed-Farid⁵, S.Hussein⁶, M.Baker⁷, O.G. Sakr⁸, M. Bionaz⁹.

¹Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt. ²Biochemistry and Biochemistry of Nutrition Department, Faculty of Veterinary Medicine, Cairo University. ³Regional Center for Food and Feed; Agriculture Research Center; Giza; Egypt. ⁴Department of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt. ⁵Physiology Department, NODCAR, Giza, Egypt. ⁶Histology Department, Faculty of Veterinary Medicine, Cairo University. ⁷Physiology Department, Faculty of Veterinary Medicine, Cairo University. ⁸Animal Production Department, Faculty of Agriculture, Cairo University, Cairo, Egypt. ⁹ Department of Animal and Rangeland sciences, Oregon State University, Oregon, USA.

Dietary conjugated linoleic acid (CLA) was reported to modify the lean mass and fat ratio in several animal species, therefore, it could be used as a functional feed ingredient to enhance the meat composition, reduce fat contents, and maximize protein contents of the meat, thus, aid in reducing the chronic cardiovascular diseases associated with high fat contents in meat. Therefore, seventy five weaned (30 days old) white New Zealand rabbits, half males half females, were used in a 63-d experiment (7 days adaptation, and 65 days experimental period). Diet was formulated to meet the growing rabbit requirements. Rabbits were blocked for body weight (612.8 ± 24.898 g) and completely randomized into three (n = 25/group) isonitrogenous, isocaloric dietary treatments: 1) group fed basal control diet (CON) supplemented with 1% oleic acid, 2) group fed diet supplemented with 0.5 % CLA, 0.5% Oleic acid (CLAL), and 3) group fed on diet supplemented with 1% CLA (CLAH). At the end of the experiment, 6 rabbits were selected, and euthanized, after evisceration, head, liver, kidney, heart, spleen, and hot carcass weight was recorded. Carcass has been cut into two equal longitudinal portions, left side of the carcass was segmented to record the mid quarter, fore, and hind limb weight. Left carcass side was then deboned and meat was minced and stored at -20 C for meat composition and fatty acid determination. Dietary CLA decreased the c181n9, and increased c182n6 in a dose dependent manner ($P \leq 0.01$). Additionally, CLA increased the meat protein percentage, and decreased fat percent ($P = 0.04$) without affecting the body weight or feed intake of the rabbits. Dietary CLA could be used to increase lean mass without any effect on feed intake and body weight in growing rabbits.

Keywords: Conjugated linoleic acid; Meat composition; Fatty acids; Growing rabbits.



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Biological activity and physicochemical quality of different types of kombucha yoghurt VS traditional yoghurt during storage

Ayah, B. Abdel-Salam¹ and Gehan, F. Galal²

¹Department of Food Hygiene & Control, Faculty of Veterinary Medicine, Cairo University, Egypt. ²Department of Microbiology, Faculty of Agriculture, Ain shams University, Egypt.

Fermented dairy products hold an important role in functional foods field which has a great interest in human nutrition. The metabolic activity of kombucha in milk and obtaining a new yoghurt type is the subject of this research. Kombucha Fermented Solutions (KFSs) were firstly produced from six different types of tea (Bergamot Tea, Camomile Tea, Green Tea, Cardamom Tea, Moringa Tea and Black Tea) and then inoculated in milk with yoghurt starter culture. Chemical composition (total solids, Ash, Protein and fat content), sensory evaluations, as well as acidity percent of produced yoghurt were measured. Biological activity of kombucha yoghurt was examined by inoculating yoghurt with *S. aureus* and tracking its viability in the product during storage. Obtained results for chemical composition showed that TS% of treatments B & C was less than that of the control. Also Fat% of treatments B, D & F was less than that of the control, while Ash% of treatment E is more than that of the control. There were significant differences in acidity percent between groups. For sensory quality, all types of produced kombucha yoghurt were accepted overall. Tracking the viability of inoculated *S. aureus* in the kombucha yoghurt showed great percentage reduction from day one especially in kombucha yoghurt with Camomile & Cardamom tea. These results encourage the availability of using kombucha yoghurt as a commercial product with high hygienic and chemical quality in addition to its acceptability for the consumer.

Keywords: Kombucha yoghurt; Physicochemical quality; *S. aureus*; Traditional yoghurt.



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Evaluation of subcutaneous infiltration of autologous platelet-rich plasma on skin-wound healing in dogs

Haithem A. Farghali¹, Naglaa A. AbdElKader¹, Marwa S. Khattab² and Huda O. AbuBakr³

¹Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt; ²Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt; ³Department of Biochemistry and Nutrition, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt

Platelet-rich plasma (PRP) is known to be rich in growth factors and cytokines, which are crucial to the healing process. This study investigates the effect of subcutaneous (S/C) infiltration of autologous PRP at the wound boundaries on wound epithelization and contraction. Five adult male mongrel dogs were used. Bilateral acute full thickness skin wounds (3 cm diameter) were created on the thorax symmetrically. Right side wounds were subcutaneously infiltrated with activated PRP at day 0 and then every week for three consecutive weeks. The left wound was left as control. Wound contraction and epithelization were clinically evaluated. Expression of collagen type I (COLI) A2, (COLIA2), histopathology and immunohistochemical (IHC) staining of COLI α 1 (COLIA1) were performed on skin biopsies at first, second and third weeks. The catalase activity, malondialdehyde (MDA) concentration and matrix metalloproteinase (MMP-9) activity were assessed in wound fluid samples. All data were analyzed statistically. The epithelization percent significantly increased in the PRP-treated wound at week 3. Collagen was well organized in the PRP-treated wounds compared with control wounds at week 3. The COLIA2 expression and intensity of COLIA1 significantly increased in PRP-treated wounds. MDA concentration was significantly decreased in PRP-treated wound at week 3. The catalase activity exhibited no difference between PRP treated and untreated wounds. The activity of MMP-9 reached its peak at the second week and was significantly high in the PRP-treated group. S/C infiltration of autologous PRP at the wound margins enhances the wound epithelization and reduces the scar tissue formation.

Keywords: Autologous platelet-rich plasma; Skin-wound healing; Dogs.



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Gastro-duodenal reflux in mongrel dogs and its histopathological findings

Naglaa A Abd Elkader¹, Kawkab A Ahmed²

¹Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary, Medicine Cairo University, Giza, Egypt; ²Department of Pathology Faculty of Veterinary, Medicine Cairo University, Giza, Egypt

The antrum and pylorus were the most susceptible portions of the stomach to any irritant. It's extraordinary movement of bile secretion through pyloric sphincter to pylorus, which called reflux gastritis. Bile acids deconjugate in pylorus to particularly harmful constituents to gastric mucosa. The recent study was carried out on twenty dogs, examined by white light endoscope and they showed evidence of gastro-duodenal reflux disease in nine dogs. Evaluating endoscopic findings in gastric mucosa especially pyloric part which associated with reflux gastritis and its histopathological findings. The endoscopic examination was carried out by using Eickemeyer video-endoscope for capturing images of different forms of reflux gastritis, followed by picking up biopsies for histopathological evaluation. There were marked detectable lesions at pylorus were detected as ulcerations, abnormal folding in pylorus, opened pyloric sphincter with bile secretion and hypersecretions alongside the gastric mucosa. The histopathological examinations of picked up 6-10 biopsies from the pyloric mucosa, revealed hyperplasia of mucosal epithelium, hyperplasia and hyperactivity of the glands, mucosal hemorrhage and marked fibrosis of gastric mucosa. The present study has proved that reflux gastritis considers hidden reason for chronic gastritis which leads to actual crisis in pylorus due to carcinogenic constituents of bile acids deconjugation.

Keywords: Gastro- duodenal; Reflux; Hyperplasia; Stomach; Dogs.



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Prevalence of intestinal coccidiosis and the associated economic losses among sheep and goats in Bahrain.

Abdalla Fadlalla Azrug¹, Eman Magzoub Saeed²

¹ Department of Parasitology, ² Department of Food Safety & Microbiology, Central Veterinary Laboratory, Agriculture & Marine Resources Affairs, P.O. Box: 251, Manama, Bahrain.

Bahrain's area-space is limited, its livestock total population is small, but it is considered as livestock importer country and the benefits gained from livestock remain of much economic impact. This study aimed to determine the prevalence of coccidiosis among sheep and goats evaluating fecal samples results records at Bahrain Central Veterinary Laboratory during 2017. There are several *Eimeria* species found in small ruminants of which the pathogenic species as *E. ovinodalis*, *E. bukensis*, *E. ahsata* in sheep and *E. christenseni* and *E. arloingi* in goats and many non-pathogenic species. Eimeriasis is more serious in ages (3-6) months lambs and kids. Samples obtained from both public veterinary services clinics and private clinics. Eimeriasis is one of the most important health problems among small ruminants with marked economic importance affecting productivity reduction, economic losses due to preventive, management and cure approaches. A total of 123 fecal samples from sheep and 47 samples from goats were received and examined for gastrointestinal helminthes and protozoans including *Eimeria* species during 2017. Modified McMaster chamber parasitic egg counting technique was performed to estimate degree of *Eimeria* infection. All the fecal samples examined in by both fecal floatation and sedimentation techniques. Thirty eight (38) sheep (30.9%) and 26 goats (44.2%) were positive with *Eimeria* oocysts. Seventeen (17) sheep (44.7%) and 11 goats (42.7%) were showed heavy infection over 10.000 oocysts per gram of faeces. Most cases were seen as mixed infections particularly with haemonchosis (61.4%). Early weaning, feed changes, intensive husbandry, bad management, and weather represent the most important risk factors associated with coccidiosis in both species.

Keywords: Intestinal coccidiosis; Prevalence and economic impact; Sheep and goats; Bahrain.



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Liver histopathology in camels (*Camelus dromedarius*) fascioliasis in Sudan

Nuseba H. Mohammed¹; Halima M. Osman² and Hind E Ahmed

^{1, 2&3} Central Veterinary Research Laboratory, P.O. Box 8067(Al-Amarat), Animal Resources Corporation, Khartoum, Sudan.

During period from 2010 – 2011 eighty camel's liver were collected from Tampool abattoir, Butana area, mid-Eastern (130 km South Eastern Khartoum), Gezira State, Sudan. Samples were examined by visualization, palpation and incisions. Grossly, these livers were firm with whitish foci scatter in the parietal surface, some were dark and swollen. Samples were taken in 10% formalin for histopathological examination. Infected livers revealed hemorrhagic hepatitis with massive destruction of liver tissues and proliferation of fibrous connective tissue replacing almost most of hepatic parenchyma with infiltration of mononuclear cells, hyperplasia of the epithelial lining of bile duct were seen in the livers. Necrosis was also noted in the hepatic epithelial layer and numerous newly formed bile ductules.

Keywords: Fascioliasis; Camel; Hyperplasia; Bile ducts.