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Feed efficiency in animal production: What is it, why do we care, and how can we improve it?

Marie E. Iwaniuk and Richard A. Erdman

Department of Animal and Avian Sciences, University of Maryland, USA

Feed is by far the single largest expense associated with producing milk on dairy farms. Feed costs currently account for approximately 50% or more of the total cost of producing milk in the United States. Because of the impact of feed cost on profitability, dairy producers are continuously exploring new methods to improve feed utilization. Feed efficiency (FE) expressed as the ratio of milk produced per unit of dry matter intake has improved dramatically over the last 50 years; primarily as a result of increased milk production per cow. The increase in production has diluted the portion of feed allocated to meet the cow's maintenance requirements from 69% to 33%. Even more dramatic changes have occurred in other livestock species such as poultry broiler production, where feed per gain has dropped from 5 to less than 2. Increased animal productivity along with reduced use of feed for maintenance of breeding stock can have profound effects of overall FE. Variation between individual animals in residual feed intake suggests that genetic selection for feed efficiency is possible. In the dairy industry, several dietary factors have been shown to improve FE. Examples include: ingredient selection to improve milk production; improved feeding management to decrease feed wastage, improved feed processing to increase feed digestibility; ionophore supplementation; and manipulation of dietary cation-anion difference. When combined in an overall management system, these changes can have dramatic effects on FE. In summary, numerous opportunities exist to increase the output of animal products with limited feed resources.

Keywords: Dairy cows; feed efficiency; nutrition.





Expected: Spring 2018

Achieved: Summer 2013

Achieved: May 2011

Current GPA: 4.00

Final GPA: 4.00

Final GPA: 3.21

Dr. Marie E. Iwaniuk brief biography: EDUCATION

Animal Science, PhD

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ACADEMIC FOCUSES

Animal Nutrition:

- <u>ANSC612</u>: Energy Nutrition
- <u>ANSC688P</u>: Protein Nutrition and Metabolism
- <u>BCHM463</u>: Biochemistry of Physiology

Statistics:

- <u>BIOM601</u>: Biostatistics I
- <u>BIOM602</u>: Biostatistics II
- <u>BIOM621</u>: Applied Multivariate Statistics

Teaching:

• ANSC688V: Introduction to University Teaching

RESEARCH EXPERIENCE

Dairy Cattle Nutrition (Dr. Richard Erdman), University of Maryland 9/2011-Present

- Developed, conducted, and analyzed two feeding experiments at CMREC
- Re-analyzed dietary buffer literature and generated surface response equations for production responses, milk composition, rumen characteristics, feed digestibility, and feed efficiency
- Assisted in milk sampling and blood collection for further analyses
- Performed statistical analyses for several graduate students within the ANSC Department

Avian Nutrition (Dr. Roselina Angel), University of Maryland 10/2009 – 9/2011 **PUBLICATIONS**

Journal of Dairy Science March 2015

• Iwaniuk, M. E., A. W. Weidman, and R. A. Erdman. 2015. The effect of dietary cation-anion difference concentration and cation source on milk production and feed efficiency in lactating dairy cows. J. Dairy Sci. 98:1950-1960.

Journal of Dairy Science

July 2015

• Iwaniuk, M. E., and R. A. Erdman. 2015. Intake, milk production, ruminal, and feed efficiency responses to DCAD in lactating dairy cows. J. Dairy Sci. 98:8973-8985.





Perfecting an animal model system for the study of human enteric viruses and vaccines

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One of the missions in Veterinary Medicine is to develop animal models of human diseases for the improvement of public health. In the past decade, our work focused on continuing efforts to establish more effective animal models for the study of human enteric viruses (rotavirus and norovirus), and for the evaluation of rotavirus and norovirus vaccines and antiviral drugs. Rotaviruses are recognized as the leading cause of severe gastroenteritis in infants and young children. After the introduction of human rotavirus vaccines in 2006, human noroviruses have become the major cause of viral gastroenteritis worldwide. Due to the lack of a robust culturing system and a suitable small animal model, norovirus vaccine development and antiviral research have long been hampered. Although mice can be infected by murine rotavirus and norovirus, they do not truthfully mimic the pathogenesis and disease symptoms seen in humans after rotavirus or norovirus infection. Neonatal gnotobiotic (Gn) pig is the only animal model that recapitulates the diarrhea disease after human rotavirus or norovirus infection. Gn pig models of Wa (G1P[8]) rotavirus or GII.4 norovirus infection and diarrhea are being continuously utilized in my laboratory at Virginia Tech for studies of pathogenesis and vaccines. The human gut microbiota transplanted Gn pig model was recently established. The model is more closely mimics the intestine environment of human infants and provides a more robust model for studies such as virus-host interaction, enteric dysbiosis and immunity; and for evaluating intervention approaches to reduce viral diarrhea. Furthermore, Gn pigs with immunodeficiency, such as Ig heavy chain knockout (lacking B cell and antibody) and CD8+ T cell depleted, were used to study correlates of rotavirus protective immunity. For norovirus, human norovirus infectivity and pathogenesis have been studied in wild type (WT), WT colonized with commensal bacteria Enterobacter cloacae, and RAG2/IL2RG deficient pigs (lacking T, B and NK cells) which were generated by CRISPR/Cas9 genome editing technology. The studies utilizing Gn pigs and genetically modified Gn pigs have improved our understanding of mechanisms of pathogenesis and immunity of human enteric viruses. Knowledge generated will facilitate the development of vaccines and antivirus strategies.

Keywords: Animal model; human enteric viruses; vaccines.





Dr. Lijuan Yuan brief biography:

Dr. Lijuan Yuan received her master's degree in virology and immunology at the Laboratory of Biochemistry and Immunology, the Capital Institute of Pediatric, Beijing, China; her Ph.D. degree in virology and immunology at the Food Animal Health Research Program, Department of Veterinary Preventive Medicine, The Ohio State University, USA in 2000 (Advisor Dr. Linda Saif). She completed her post-doctoral training at the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), Bethesda, USA in 2002. She was a Research Scientist and Adjunct Assistant Professor at the Department of Veterinary Preventive Medicine, The Ohio State University from 2002-2007. From July 2007-2013, Dr. Yuan was an Assistant Professor of Virology at the Department of Biomedical Sciences and Pathobiology at Virginia Polytechnic Institute and State University, Blacksburg, Virginia. She received tenure and was promoted to Associate Professor in June 2013. Dr. Yuan has published 94 scientific publications, including 69 peer-reviewed journal articles, 10 book chapters, and 15 reviews. Dr. Yuan's research has received national and international recognition. She serves on many NIH, USDA, NSF, Wellcome Trust, Qatar National Research Foundation, National Natural Science Foundation of China and other international grant review panels. She received the International Professorship award from American Society for Microbiology and was awarded the title of Honorary Professor by Yunnan Agricultural University, China in 2009. She was the recipient of the Pfizer Award for Research Excellence in 2011. Her laboratory's research projects are funded by NIH, the Bill and Melinda Gates Foundation, PATH, and vaccine companies. She has been awarded 13 research grants as a principal investigator totaling more than 5.5 million U.S. dollars and served as the major professor for 7 PhD students and on the graduate advisory committee for 24 other students.





Describing some behavioural animal models of anxiety and their mecanistics. Oxytocin relevance?

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In the present mini-review we will try to summarize the current knowledge and our previous experience in dealing with animal models of anxiety, by focusing on various species such as rats, zebrafish, dogs or pigs, as well as describing the relevance of oxidative stress in this context. In addition we will shortly describe the possible relevance of the oxytocin administration in this context, considering its increased relevance in this area of research in the last couple of years.

Keywords: Animal models; anxiety; oxytocin.





Dr. Alin Ciobica brief biography:

- Short training stage (December 2007) in Dept. of Molecular Biology of Umea University (Sweden) – Prof. Anna Berghard and Prof. Staffan Bohm (collaborators of Linda Buck, Nobel Winner-2004).
- Short Scientific Visit (June 2013) in Department of Psychiatry, Osaka University, Gradutate School of Medicine, Osaka, Japan.- Prof. Masatoshi Takeda and Prof. Takashi Morihara.
- December 2013 Dr. Marin Kolisek and Prof. Jörg R. Aschenbach, Institut für Veterinär-Physiologie, Freie Universität Berlin developing and studying a new rat model of Parkinson's disease, based on the possible deficiency of intracellular magnesium.
- January 2014 Prof. Dr. Hafedh Abdelmelek- Faculty of Sciences de Bizerte (Tunisia) – establishing further possible collaborations regarding behavioral studies and oxidative stress status in rats, by using European-African collaboration funds.
- March 2014 Prof. Dr. Karl Ægir Karlsson- Reykjavik University setting the basis for further collaborations regarding the performing of some behavioral tasks in zebrafish, Parkinson's disease-related studies and models in zebrafish or drug screening in sleep-related zebrafish studies (e.g. Angiotensin-1-7).
- September 2014 Prof. David Grey's group from the School of Physiology, Wits University in Johannesburg, South Africa --- 45 minutes oral presentation and especially establishing a future grant/collaboration with Prof. Neville Pitts and Dr. Dee Muller on the relevance of some biomarkers like cortisol or oxidative stress markers in some neuropsychiatric disorders, with special focus on children and/or autism.
- H-index: 18, with more than 350 ISI citations (excluding self-citations) for the papers published in the last 4 years.
- Reviewer for more than 30 ISI Journals including: Journal of Alzheimer's Disease, Schizophrenia Research, Progress in Neuro-Psychopharmacology & Biological Psychiatry, Journal of Psychiatric Research, Psychiatry Research, Psychiatry and Clinical Neurosciences, Neurochemical Research, Chemosphere etc.
- ISI-indexed publications (more than 60 ISI articles). Do not include publications submitted or in preparation.





The role of Veterinary Medicine at the sustainable development of

livestock in Egypt

Dr. Youssry A. Radwan Livestock and Animal Health Consultant Expert-Egypt & GCC Countries

Livestock play multiple roles in developing communities, they provide food and nutrition, work, economic and social status, and ensure environmental sustainability. With the livestock sector experiencing rapid change - mainly driven by the rapidly changing livestock production systems, demographics, environmental impacts, technologies, policies, and institutions – this 'multifunctionality of livestock' becomes an even more complex issue, intertwined with other research and development challenges. The multifunctional capacities of food animals are of four categories. They (i) provide a means of diversifying the use of resources and reducing socio-economic risks, (ii) promote linkages with other systems' components, (iii) generate value-added products such as milk and meat, and provide attendant services such as drought power, and (iv) contribute to sustainable livestock development. Livestock intensification is a response to increased demand for livestock products, especially meat, milk and eggs. Although intensification offers opportunities for better income, it may deny smallholders the benefits of the multifunctionality of livestock, particularly the intangible benefits derived when products become increasingly commodifized. Livestock intensification is bringing about structural changes in livestock system, particularly within dairy cattle and buffaloes, as well as, sheep and poultry subsectors which provide huge returns per until input and offer breeders economies of scale. To date, revitalised agenda for development is wanting, given that livestock constitutes about 30 per cent of the agricultural gross domestic product (GDP) in the developing world. It is also one of the fastest growing subsectors in agriculture. Livestock provide a variety of food and nonfood products; the latter include leather, wool, pharmaceuticals, bone products, industrial protein, and fats. However, economic implications of sustainable development of livestock extend further: to downstream industry, upstream industry and associated services.

Keywords: Economic and social status; Livestock; Sustainable development.





Dr. Youssry A. Radwan brief biography:

- <u>Scientific Qualifications:</u>
 - B.V.Sc (1972), Faculty of Veterinary Medicine, Cairo University, Egypt
 - M.V.Sc (1976), Internal Medicine, Faculty of Veterinary Medicine, Cairo University, Egypt
 - Ph.D (1979), Internal Medicine and clinical Laboratory Diagnosis Faculty of Veterinary Medicine Cairo University, Egypt
- <u>Academic Status Record:</u>
 - 1972-1982: Instructor, Assistant Lecturer, Lecturer, Assistant Professor, Faculty of Veterinary Medicine, Cairo University, Egypt.
 - 1983-1985: Head of Department of Veterinary Medicine & Surgery, Faculty of Veterinary Medicine, Tripoli Libya.
 - 1986-1991: Assistant Professor, and Full Professor, Department of Medicine and Infectious Diseases Faculty of Veterinary Medicine, Cairo University, Egypt
 - 1992-2004: Professor of Medicine and Therapeutic, Clinical Studies Department, College of Veterinary Medicine and Animal Resources, King Faisal University, Saudi Arabia.
 - 2005-2014: Animal Health Consultant Expert, Ministry of Municipality, Planning, Environment, Doha Qatar
 - 2014-2015: Livestock and Animal Health Consultant Expert, UrbaCon Trading & Contracting International Company-Doha Qatar.

<u>Present Position:</u> Livestock and Animal Health Consultant- Expert (**Between Egypt** & GCC Countries - Private Consultant Office)

- Publications:
 - Published Scientific Research- (50).
 - Cultural and Scientific Books- (13).





Recent vaccination strategy for controlling of ILTV

Ahmed Adel Seida

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Infectious Laryngotracheitis (ILT) is a viral infection of the respiratory tract of chickens. The disease is seen in layers, breeders and in some areas in broilers as well. It spreads rapidly from bird to bird and can cause high mortality rates.

ILT is present in many countries around the world and remains a major threat to all poultry producers. Outbreaks of mild to moderate forms of ILT are common in commercial layer flocks, while sporadic outbreaks of ILT in broiler flocks have been recognized as an emerging problem in many countries.

In countries where ILT is present, layer flocks are vaccinated with one or two doses of modified live virus vaccine.

There are two types of modified live-virus vaccines: CEO (Chicken Egg Origin) vaccines, and TCO (Tissue Culture Origin) vaccines. CEO vaccines may cause post-vaccination reactions, characterized by mild to moderate respiratory symptoms.

A known feature of ILT viruses is that they may persist or cause latency in chickens which are then capable of shedding the virus to susceptible chickens.

Innovax® ILT is an innovative vaccine from MSD Animal Health that protects against ILT and MD. Innovax® ILT is based on a HVT vector in which two immunogenic glycoprotein genes of ILT are inserted. The HVT was chosen as the vector since it is recognized as a safe virus to chickens and stimulates long-lasting immunity against MD and ILT.

One-shot – life-long protection, just one dose of Innovax® ILT vaccine applied in the hatchery to day-old chicks or in-ovo to 18-day embryos results in long lasting protection against both ILT and MD. Protection lasts for at least 60 weeks for ILT.

Keywords: Chickens; ILT; Infectious Laryngotracheitis; vaccine.





Optimum crude protein requirement of the Nile Tilapia (O. niloticus)

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Fingerlings of tilapia (*O. niloticus*) were fed four isocaloric diets with different crude protein levels to determine the optimum protein requirement of the species. Eighty of apparently healthy fingerlings tilapia (*O. niloticus*) fish, of average weight 10 g were divided into four groups, each of 20 fish. Four experimental diets were formulated with four levels of dietary protein 27, 30, 33 and 36% and constant digestible energy, 3000 kcal/kg diet. The experiment lasted for 10 weeks, in addition to 2 weeks before starting used as an adaptation period to accustom the fish to the diets and the environmental conditions. Significant differences (P<0.05) were recorded for the growth indices, weight gain and feed conversion and the highest values obtained for the fingerlings fed the 33% crude protein diet. Crude protein, fat and ash contents of tilapia's body were not significantly different among the fish fed the diets containing different protein levels. Protein retention, protein in gain and protein efficiency ratio of the fish fed diet containing 33% dietary protein were significantly (P<0.05) higher than other groups. It is therefore, concluded that 33% crude protein is optimal requirement in the diet of fingerlings of tilapia (*O. niloticus*).

Keywords: Crude protein; tilapia; requirement.





In vitro activity of some essential oils alone and in combination against

the fish pathogen Nocardia seriolae

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Microplate resazurin assay was applied to investigate the *in vitro* activity of four essential oils (EOs); cinnamon (Cinnamomum zeylanicum), thyme (Thymus vulgaris), lemongrass (Cymbopogon flexuosus) and tea tree (Melaleuca alternifolia) oils against 80 clinical isolates of the fish pathogen Nocardia seriolae. Afterwards, the checkerboard test was used to determine the possible synergistic effect of EOs combination against reference type strains of fish nocardiosis. All tested EOs had antibacterial activity against N. seriolae isolates. Among the tested EOs, cinnamon and thyme oils both exhibited the lowest minimum inhibitory concentrations (MICs) with 5-160 and 10-160 µg/ml, respectively. The activities of lemongrass and tea tree EOs were noted to be less effective with MICs of 20-640 and 160->5120 µg/ml, respectively. The checkerboard panel of cinnamon-thyme EOs combination against N. seriolae ATCC43993 demonstrated a synergistic effect with fractional inhibitory concentration (FIC) index of 0.75. For N. salmonicida ATCC27463, the combination panel showed an additive effect with FIC index of 1.0. For N. asteroides ATCC19247, the combination panel demonstrated an indifference effect with FIC index of 1.125. These results indicate that thyme and cinnamon oils alone or the combination of them at a given ratio has a promising potent clinical significance in the treatment of fish nocardiosis. Despite the promising results given by our *in vitro* studies, the clinical benefits of these EOs combinations can only be determined through carefully designed in vivo experimental studies.

Keywords: Cinnamon; essential oils; MIC; Nocardia seriolae; thyme.





Eco-toxicological and bactericidal effectiveness evaluation bioassay of some commercial disinfectants in aquaculture

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Disinfectants are vital tools for effective fish farm biosecurity; however, eco-toxicological data about chemotherapeutic use in aquaculture are not available or difficult to be obtained. In vivo evaluation study was necessary for establishing whether a previous in vitro bactericidal effectiveness evaluation of selected six commercial disinfectants was assessed by the proposed CEN 1276 Phase2 step1 test that was followed, beside investigation the safety application of the tested disinfectants into an aquatic system. In a three sequential studies, the evaluation bioassay of six commercial disinfectants, Virkon-S[®], Biosentry[®] Iodine[™], Biosentry[®] 904[™], Aldekol des-Gda[®], TH4[®] and Peraclean[®], was conducted at No Observed Effect Concentration (NOEC) after acute and chronic disinfectant toxicity testing following OECD (guideline no.203) challenged against three common bacterial genera causing disease problems in aquatic ecosystem, Aeromonas hydrophila (gyr-B LC012344), Pseudomonas aeruginosa and Vibrio alginolyticus, at median infective dose (ID_{50}) obtained from infectivity test. Efficient bactericidal effectiveness was confirmed by Virkon-S[®], Aldekol des- Gda[®] and Peraclean[®] at NOEC against the challenged bacterial strains, on the other hand TH4[®] disinfectant failed to achieve the bactericidal effect, in spite of its proven efficient bactericidal reduction under *in vitro* conditions against all challenged bacterial strains in a previous study, meanwhile, eco-toxicological data classified biosentry[®] 904[™] as classI, acute toxic disinfectant, while Virkon- S[®], Aldekol des- Gda[®], TH4[®] and peraclean[®] as class III, slightly toxic chemicals as LC_{50} / 96 hours values were 100, 50, 35, 100 ppm, respectively. So, Virkon-S[®], Aldekol des- Gda[®] and Peraclean[®] were of wide safety margin and can be used for disease control in aquaculture achieving efficient bactericidal effectiveness in contrary to TH4 that failed to achieve in vivo bactericidal effectiveness therefore, the standardized steps for testing disinfectants under both in vitro and in vivo should be followed to achieve complete evaluation data.

Keywords: Acute and chronic fish toxicity testing; aquaculture; disinfectant testing; eco-toxicology; *in vivo* evaluation; OECD 203 test





Protective Effects of copper-nicotinate complex against nephrotoxic effect of glycerol in rats

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This study was carried out to investigate the protective effect of short-term exposure to copper-nicotinate complex against glycerol in rats. Kidney function tests, oxidative cascade & histopathology in kidney were evaluated. Forty albino Swiss rats were randomly distributed equally into four groups; the control one. The second group was given cu-nicotinate complex orally by stomach tube three times per week for continuously four weeks. The third group was given glycerol as topical application daily for continuously four weeks. The fourth group was given cu-nicotinate complex with glycerol for the same period. Glycerol-treated rats suffered from depression and lowered activity. There was marked increase in urea & creatinine at the end of the experiment. A marked increase in lipid peroxides associated with a decrease in reduced glutathione, SOD and CAT activity were observed in liver homogenate of the glycerol-exposed animals. Renal casts, necrotic changes in renal medulla, perglanular fibrosis with infiltration of inflammatory cells associated with degenerative changes in renal tubules were observed in group treated with glycerol. Where the group treated with coppernicotinate complex together with glycerol was showed normal level of urea & creatinine. A marked decrease in lipid peroxidation associated with an increase in reduced glutathione, SOD and CAT activity were recorded. Histopathology revealed normal structure of renal tubules in the fourth group. In conclusion, cu-nicotinate complex can be used to protect the kidney against the degenerative changes caused by glycerol.

Keywords: Copper-nicotinate complex; glycerol; rats.





Using of glucan and flagellin as adjuvants for prepared inactivated

rabies vaccine

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Vaccination is the most effective method for preventing rabies virus infection in both humans and animals; however, no satisfactory vaccine has been developed for use worldwide. So, the main goal now is to improve the present produced inactivated cell culture rabies vaccine. In the present study, we investigated the immunoadjuvant properties of β (1, 3) D-glucan and *Salmonella* Typhimurium flagellin (FliC) to improve immune responses of dog to the inactivated rabies vaccine. Administration of glucan (group-1), flagellin (group-2) and aluminum hydroxide gel (group-3), in the form of adjuvants to puppies (aged 3-4 months) via inactivated cell culture rabies virus vaccine showed marked increase in protective level of rabies antibody titers in case of using glucan and flagellin than those vaccinated with traditional produced vaccine using aluminum hydroxide gel. The highest level of rabies serum neutralizing antibody titers were (83) and (80) in 28 days post-vaccination of puppies in case of using glucan and flagellin adjuvanted inactivated rabies vaccines respectively. Contrary to that, puppies were using of aluminum hydroxide, induced the lowest rabies antibodies titers (32) in the same period. We can conclude that glucan and flagellin have relevant immunostimulatory effects on immune response of dogs to inactivated cell culture vaccine than those exhibited with aluminum hydroxide gel rabies vaccine.

Keywords: Adjuvant; β (1, 3) D-glucan; inactivated rabies virus vaccine; *S*. Typhimurim flagellin.





Molecular characterization of recent isolates of BEF virus in Egypt

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The present work was conducted on 103 cows from total 1600 vaccinated cow reared in El-Salhia dairy farm during the period of July and August 2015. The animals revealed clinical signs lead to suspect infection with bovine ephemeral fever as fever; harried respiration; lameness and recumbence. The 103 animals were found to have nonprotective level of BEF antibodies ranged from 0- 1/2 when tested by serum neutralization test (SNT). 103 serum samples, three buffy coat and five blood plasma samples from the 103 suspected infected dairy cattle were used for identification and characterization of recent isolates of bovine ephemeral fever virus (BEFV) in Egypt. Trials of virus isolation in baby mice brain and BHK₂₁ cell culture revealed that one buffy coat sample and one blood plasma sample of the same animal were suspected to be BEFV showing specific signs in inoculated mice (paralysis of the limbs, nervous symptoms and death within (3-4) days post inoculation) and specific cytopathic effect in BHK-21 cell culture. BEFV identification by two ways firstly by direct fluorescent antibody technique (FAT) and confirmed by polymerase chain reaction (RT-PCR), Sequencing analysis and phylogenic tree showed that the obtained isolate has identity to bovine ephemeral fever virus isolate EGY-2005 by 90 % and with bovine ephemeral fever virus isolate EGY 12 glycoprotein by 88%. Finally we concluded from these findings: the used vaccine is highly immunogenic inducing a protection rate of 93.6%. Appearance of disease signs on cow with low immune levels could be due to parturition and high lactation stress factors in addition to suspected other infection leading to poor immune response (immune suppression) to the vaccine.

Keywords: BEFV isolation; Identification; PCR; FAT.





The effect of multiple passages of Egyptian HPAIV H5N1 on MDCK cell line on the virus titer and mutation of HA gene

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Highly pathogenic avian influenza viruses subtype H5N1 continually circulated in Egypt. Hemagglutinin (HA) of influenza virus mediates both receptor binding and membrane fusion. Well-established mammalian cell substrates such as MRC-5, MDCK or VERO have been used for many years for viral vaccine production. In this study 15 isolates of HPAIV were passaged in MDCK cell line for ten serial passages. The possibility of incidence of mutation in HA gene of the tested viruses after these passages were studied. Infectivity, quantitative real time PCR and HA activity indicated the increase of virus yield after passaging on cells. Sequencing of HA gene revealed no evidence of any mutation in the HA gene after five or ten passages. Antigenic characterization of these viruses indicated there is no any antigenic variation between the parent viruses and produced viruses after 5th and 10th passage. Thus, results proven a potential MDCK cell line for H5 subtype avian influenza virus propagation and application for this cell line in cell based influenza vaccine production in the poultry field.

Abbreviations: MDCK: Madine Derby Canine Kidney, HPAIV: Highly pathogenic avian influenza viruses, PCR: Polymerase chain reaction.

Keywords: Antigenic characterization; Highly Pathogenic Avian Influenza Virus; MDCK; rRT-PCR; Sequencing.





Genetic analysis of HA, NA and NS genes of avian influenza viruses subtype H9N2 isolated from chicken and quail in Egypt

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Avian influenza virus subtype H9N2 has become established in poultry in Egypt since 2011 causing serious threats when associated with other pathogen infections. In this study, we investigated the genetic characterization of HA, NA and NS1 genes of two H9N2 viruses. Phylogenetic analysis of both viruses showed that they are reassortant possessing 1 gene segment (NS1) related to subtypes H6N2, H2N3, H7N10 and H3N8 isolated from wild birds; HA and NA genes were from the subtype H9N2 G1 lineage circulated in the Egyptian viruses.

The important changes were found in the H9N2 virus isolated from quail as the deduced amino acid sequences of the hemagglutinin (HA) gene showed the presence of new glycosylation site at position 206, in addition to five amino acid substitutions detected in the receptor binding domain and antigenic sites. The Hemagglutinin cleavage site motif is PARSSR which indicates the low pathogenicity nature of these viruses. Genetic analysis of neuraminidase gene showed the presence of new glycosylation site at position 306 in the quail virus. The PDZ ligand (PL) motif at the C-terminus of NS1 protein of our isolates is ESEV which is typical for avian species. The results from this work indicate the importance of continuous monitoring of viral genetic changes especially in endemic countries like Egypt to update the epidemiological situation and that will help the efforts to control.

Keywords: Avian Influenza Virus H9N2; Egypt; genetic analysis.





Some markers in Pb1, Pb2 genes of H5N1 of samples from 2010 till 2015

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Clade 2.2 H5N1 highly pathogenic avian influenza viruses (HPAIVs) have been in continuous circulation in Egyptian poultry since 2006. Their persistence caused significant genetic drift that led to the reclassification of these viruses into subclades 2.2.1 and 2.2.1.1,

In this study; ten Egyptian H5N1 viruses collected from apparent healthy chickens and ducks during 2010-2015, the nucleotide sequences of the (PB1, PB2) gene were amplified by RT-PCR and sequenced. The Phylogenetic relationships and mutations analysis was done. Phylogenetic analysis of these viruses revealed a close relationship between Egyptian H5N1 viruses to viruses isolated from neighboring Middle Eastern countries. Importantly, in this study we find that all samples contain (PB2 627K), a known mammalian adaptation motif. Previous avian influenza virus isolates have carried glutamic acid in this position (PB2 627E), it is through that the viruses that carry motif (PB2 627E), glutamic acid residue at 627 restrict viral growth in avian but change to lysine (E627K) restores replication in human. There are some other changes in PB1-F2 in amino acid. The substitution N66S that increases virulence detected in Egyptian viruses, The mammalian-host-associated substitution 73E and L82S in PB1-F2 was also identified in all of the viruses under study, some other mutational changes in PB 1 also detected in all classic strains, the variant strain contain some specific changes R584H, N645S, R430K. In light of our findings, H5N1 in Egypt continues to evolve; surveillance and molecular studies are needed to be sustained.

Keywords: H5N1; polymerase basic 1, 2 (PB1, PB2); PB2 627K N66S; rT-PCR; mutations analysis.





Mutation Signature in Nucleoprotein gene of Avian Influenza LPAI-

H9N2-G1 Proposed Predilection for Mammalian Adaptation

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²VirologyDept, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt, 12211 The low pathogenic avian influenza H9N2 viruses have become enzootic in Eurasia. In Egypt (LPAI/H9N2/G1) became the most prevalent and widespread causing respiratory problems and economic disasters. Recently became transmitted to humans. LPAI-H9N2 virus has segmented negative sense RNA genome; each single-strand RNA encapsidated with nucleoprotein (NP). The NP plays important role in virus genome transcription, replication and packaging beside its important role in mammalian adaptation and interspecies transmission of virus.

In this study; seven Egyptian H9N2 viruses were collected from apparent healthy chickens and quail during 2014-2015, the complete nucleotide sequences of the nucleoprotein (NP) gene were amplified by RT-PCR and sequenced. The Phylogenetic relationships and mutations analysis was done. Phylogenetic analysis of these viruses revealed a close relationship between Egyptian H9N2 viruses to viruses isolated from neighboring Middle Eastern countries, especially Israeli with an average of 96–99 % homology sharing the common ancestor A/Q/HK/G1/97(G1-Like) with no new evidence of reassortment. The H9N2 viruses in Egypt showed wide spread occurrence in the country without restrictions to specified geographical distribution. Three markers which affect the virus replication and transmission in mammalian hosts were identified especially the substitution of Isoleucine (I) by vallin (V) at position109 (I¹⁰⁹V). These special features of nucleoprotein (NP) of LPAI-H9N2 viruses refer to the tendency for enhanced introductions into humans and ensuring the importance of poultry in the transfer influenza viruses.

Keywords: G1-LIKE; LPAI-H9N2; mutations analysis; Nucleoprotein (NP); phylogenetic; RT-PCR.





Pathotyping of different Egyptian Variants of Infectious Bronchitis Viruses

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Infectious bronchitis (IB) is one of the important viral diseases of chickens, and in spite of regular vaccination, IBV is a continuous problem in Egyptian poultry operations. Herein, seven IBV strains isolated during 2012 to 2015 representing the different variants circulating in Egypt (Egy Var 1- Egy Var 2 - 793B - D274 Serotypes) were used for pathogenicity assessment in one-day SPF chicks to determine whether the reported disorders observed in the samples obtained from the different field poultry sectors were caused by an IBV strain or as a complication with other pathogens. The pathogenicity ranged from 30% to 70% with sever microscopic lesions in the kidney. In contrary, the isolates representing the two vaccinal strains 793B and D274 serotypes cause 0%, 10% mortality, respectively. Further epidemiological surveillance studies are needed in order to explain the mechanism of emergence of variants and their biological properties, including pathogenicity and vaccine trails to help in disease control.

Keywords: Egypt; histopathology; Infectious bronchitis virus; pathotyping; variant IBV.





Study on cross neutralization between various classic and variant vaccinal strains of infectious bronchitis and Egyptian variant isolates Ekram S.Mahmoud¹, Nermeen Mahmoud²,Ghada M. Elsadek³, Hyam farok⁴, Lamiaa M. Omar ⁵and Abdel Baky , M.H⁶.

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Infectious Bronchitis Virus (IBV) is considered an important virus which may cause major losses in the poultry industry. One of the most important and effective tool that control the IB infection and spread is the vaccination strategy. Many types of IB vaccines were used in Egypt, containing different vaccinal IB strains either classical or variant. Recently, in Egypt, local variant IB strains have been isolated and cause losses in some poultry flocks. The aim of this study was to determine the cross neutralization between the classical and variant vaccinal strains of IB against the local circulating variant viruses for protection of chicken. A group of SPF chicken were vaccinated with live MA5,4/91,CR88,and D274 IB vaccines and another group was left unvaccinated as control group. Three week post vaccination, all the vaccinated and control group were bled for serological analysis by HI test using the variant IB and classical antigens. Also positive serum samples against IB strains prepared in rabbits were examined by HI test against classical positive antigens. The data observed that all types of the tested serum samples either from chicken or rabbits gave higher titre against classical IB antigen than that against variant antigen. Also, it was noticed that the classical vaccinal IB strain vaccines gave higher antibody titers than variant strains and the classical IB antigens is more effective in determine antibody titer than variant one.

Keywords: Egypt; Infectious bronchitis virus; Vaccine.





Genotyping of Newcastle disease virus (NDV) isolated from Egyptian field strains using Pyrosequencing

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Newcastle disease (ND) is a highly contagious avian disease, that poses considerable threat to the poultry industry worldwide, Newcastle disease virus (NDV) is a member of serotype 1 avian paramyxoviruses (APMV-1) that causes respiratory and neurological disease in chickens and other species of birds. In this study a method based on RT-PCR and pyrosequencing analysis has been developed, to diagnose rapidly and pathotype NDV directly in clinical specimens, A pair of degenerated primers was designed to amplify a portion of the fusion (F) gene responsible for virulence and used to test 14 specimens collected from Egyptian farms in 2013 and 2014 . The subsequent pyrosequencing reaction identified a 30-bp region encompassing the cleavage site. All samples were pyrosequenced and results were compared and confirmed by the Sanger sequencing procedure, which is traditionally performed for NDV pathotyping.

The pyrosequencing reaction provided high quality results in real time and proved to be more rapid and cost-efficient than the classical sequencing procedure, indicating it as a possible valid alternative to the currently used diagnostic assays for NDV.

Keywords: Fusion gene (F gene); NDV; pathotyping; pyrosequence; RT-PCR.





Preparation and evaluation of chemically inactivated *Salmonella* Enteritidis vaccine in chickens

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Salmonella Enteritidis Bacterial ghosts (BGs) Vaccine candidate protective immunity. Salmonella Enteritidis ghosts (SEGs); non-living empty bacterial cell envelopes were generated by using the minimum inhibitory concentration (MIC) of sodium hydroxide (NaOH) and investigated as a vaccine candidate in SPF chicks. To determine the immunogenicity and protective efficacy of SEG vaccine, SPF chicks were divided into three groups: group A (non-vaccinated control), group B (s/c vaccinated with prepared vaccine) and group C (s/c vaccinated with commercial vaccine). Vaccination of SPF chicks with SEGs induced significant immune responses before and after virulent challenge. SPF chicks vaccinated with SEGs showed significant increases in serum IgG antibodies after challenging with virulent *S*. Enteritidis on week 4. During the vaccination period, groups B and C showed higher serum bactericidal activity (SBA) compared to group A. In conclusion, these results show that the chemically induced SEGs as a vaccine candidate against virulent challenge.

Keywords: Evaluation; Chicken; Salmonella Enteritidis vaccine.





Detection of some virulence genes in *Salmonella* Kentucky isolated from poultry.

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Salmonella Kentucky has an increasing world wide spread among human and animals which poses a great problem. Twenty six *Salmonella* Kentucky strains isolated from different samples from field cases between 2011 till 2016, twenty two from chicken and four from quail. In the present work we study the genetic diversity through screening of 11 virulence genes (*invA*, *avrA*, *ssaQ*, *mgtC*, *siiD*, *sopB*, *gipA*, *sodC1*, *sopE1*, *spvC*, and *bcfC*) by PCR. The *invA* gene was detected in 100% of the *Salmonella* strains; but 88.4% of strains were carry *SopB*, *avrA*, *bcfc* and *ssaQ*, *mgtc* (80.7%), *sopE* and *sodc* (19.2%), *Siid* (11.5%), *spvc* (3.8%), while no one carried *gipA*. These results showed the presence of virulence genes in *Salmonella* Kentucky with potential poultry and public health hazard.

Keywords: avrA gene; Salmonella Kentucky; ssaQ gene.





Characterization of some aerobic bacteria isolated from commercial Quail flocks

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Quail production represents a promising source to cover the deficit in the animal protein in Egypt. However, little is known about the prevalence and antibiotic resistance of major bacterial pathogens in quail farms. A total one hundred swabs and 600 organs samples have been collected from apparently healthy and freshly dead quails respectively. Bacterial isolation and characterization were performed in accordance with the clinical laboratory standards and confirmed by PCR. Antibiotic resistance tested using stander disk diffusion. In life quails only E. coli and Salmonella could be recovered from Cloacal swabs, while in freshly dead birds all four pathogens disseminated in various organs with higher incidence in liver and heart and higher incidence of mixed compared to single infection. E. coli (O78) and S. Enteritidis could be recovered mainly from heart and liver of freshly dead birds. The recovered E. coli, S. aureus and P. haemolytica isolates showed (57.1-100%) resistance to highly important antibiotic groups (Doxycycline, Tetracycline, Trimethoprim sulfa methoxazole and Chloramphenicol) and dissimilar pattern of resistance to critical important antibiotic group. Salmonella isolates showed antibiotic resistance to Nalidixic acid (100%) and Nitrofurantoin (42.9%). Strict biosecurity measures were required to reduce the incidence of mixed bacterial infection and control spread of antibiotic resistance between bacterial spp.

Keywords: Antibiotic resistance; E. coli; Pasteurella; quails; Salmonella; S. aureus.





Phenotypic and genotypic characterization of *Listeria* species isolated from different types of poultry eggs.

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The study aimed to investigate the contamination of eggs by Listeria monocytogenes and other *Listeria* spp. the causative agent of fatal food poisoning and to study the antibiotic resistance attributes of isolates. Out of total examined 400 eggs from different poultry species (100,100,100, 50 and 50) from chicken, ducks, turkey, geese, and pigeons respectively, Listeria spp. were detected by using conventional methods and PCR for (detecting 16SrRNA gene) in 58 (14.5%) and 47(11.8%), respectively. Listeria monocytogenes specific hylA gene was detected in 5.5% of eggs samples, the key virulence regulator prfA gene was detected in 13 (27.3%) of Listeria strains. Studying antibiotic susceptibility profiles of Listeria strains by disc diffusion against 12 chemotherapeutic agents revealed that all isolates (100%) demonstrated resistance to ampicillin. Furthermore, antibiotic resistance rates of 93.6%, 87.2%, 87.2%, 80.9%, and 74.5% were detected against erythromycin, trimethoprim, ciprofloxacin, levofloxacin and gentamicin, respectively. Rating antibiotic resistance categories of isolates revealed that 41 (87.2%) of isolates demonstrated multidrug resistance, while 6(12.8%) isolates demonstrated extensive drug resistance. Investigating the genotypic attributes for quinolone resistance revealed that 15 (31.9%) of *Listeria* isolates carried parC gene, while gyrA gene was not detected among the studied isolates. Resistance against Betalactam antibiotics was studied by PCR and confirmed that 19 (40.4%) of isolates carried *bla*^{tem}gene for penicillinase. The findings highlighted the significant public health risk imposed by the circulating *Listeria* strains that contaminate household eggs which carried both virulence attributes and antibiotic resistance genes, thus routine monitoring programs for egg production systems are needed with special reference for implementing HACCP and food safety systems to mitigate the consequent potential biological public health risk.

Keywords: Antibiotic resistance; Eggs; Listeria species.





Prevalence and antimicrobial resistance of *Salmonella enterica* and *Listeria* spp. in food samples collected from Sharm El sheikh, Egypt.

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The present study aimed to analyze the prevalence and antimicrobial resistance of major microbes of food poisoning in Sharm El Sheikh City, Egypt. 184 food samples were examined to evaluate the prevalence of *Salmonella enterica* and *Listeria* spp. isolation of both species was done using the methods described by the International Organization for Standardization. Obtained isolates were further examined for antibiotic sensitivity. Results showed absence of detection of *Listeria spp*. Among all examined food samples whereas, 7 out of 184 food samples were found positive for *Salmonella enterica* with a percentage of 3.8 %. Among the studied food samples, chicken based food samples showed lower prevalence of *Salmonella enterica* than red meat based samples with percentage of (3.6%) and (6.25%) respectively. Vegetable based cooked food samples were found free from both *Salmonella enterica* and *Listeria* spp..

In conclusion, this work revealed low prevalence of food borne organisms including *Salmonella enterica* and *Listeria* spp in different food samples obtained from Sharm El Sheikh which reflect high level hygiene and good quality of food products in Sharm El Sheikh. Continued surveillance of the prevalence of food borne pathogens is recommended.

Keywords: Antimicrobial resistance; food; Egypt; *Listeria* spp.; *Salmonella enterica*; Sharm El- sheikh.





Morphological characterization of the laryngeal mound of the Egyptian

geese

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Our study was carried on thirteen adult healthy Egyptian geese (*Alopochen aegyptiacus*) of both sexes. The samples were processed to study the morphological characters of the laryngeal mound grossly, histologically and by scanning electron microscopy. Morphologically, the laryngeal mound was oval elevation carried dorsally the glottis that continued caudally with the laryngeal sulcus, which surrounded on each side by conical-shaped papillae with pointed apices; some of them had broad roots. Histologically, the laryngeal mound was lined by respiratory epithelium; pseudostratified columnar ciliated type with goblet cells and intraepithelial glands. There are two types of intraepithelial glands; serous and mucous types. The mucous one lined by GAG producing cells, while the serous one lined by four different cells; GAG producing cells, large faintly stained granules cells, small dark blue stained granules cells and progenitor cells. The current study described the macro-and micro-scopical features of the laryngeal mound of the Egyptian geese.

Keywords: Egyptian geese; laryngeal mound; morphological characterization.





Effect of dietary induced negative energy balance on mammary gland lipogenic activity in mid lactating Holstein dairy cow.

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Most milk components are relatively stable under different dietary and physiological conditions; however, milk fat varies greatly. We hypothesized that dietary feed restriction can be used as a tool to study the shift in milk fatty acid profile. Ten multiparous, mid-lactating Holstein dairy cows were used in completely randomized repeated measures design. The cows were fed ad-libitum the basal diet for 14 days, followed by a 4 day restriction period in which 5 cows fed ad-libitum and the other 5 cows were fed only 60% of their ad-libitum intake, and finally, 3 days of post-restriction all cows were fed ad-libitum the same basal diet to follow up post restriction changes. Milk and blood samples were collected on the last day of each period and processed for fatty acid analysis. In the feed restricted cows, milk fat percentage tended to increase; however, milk and milk fat yield significantly (P < 0.05) decreased. Additionally, the serum non-esterified fatty acid (NEFA) concentration increased (P < 0.05) and the increased serum NEFA was associated with a significant (P < 0.05) reduction in fatty acids C4:0 to C14:0 that are synthesized in the mammary gland and a significant reduction in C16:0 which is partially synthesized in the mammary gland. In conclusion, the results of this study indicated that the level of feed intake could affect mammary lipogenesis; therefore, dietary-induced negative energy balance can be used as a model to study the nutritional regulation of mammary lipogenesis.

Keywords: Dairy cow; feed restriction; mammary lipogenesis.





Molecular detection of the clumping factor (fibrinogen receptor) in the enterotoxigenic *S. aureus* isolated from raw milk and traditional

Cheese

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The objective of this study was to determine the occurrence of enterotoxigenic *S. aureus* within 205 raw milk and traditional cheese samples examined. The *S. aureus* strains isolated from raw milk and traditional cheese samples examined were 50 (58.8%, 50/85) and 55 (45.8%, 55/120) respectively however both camel milk and cream cheese samples had not any *S. aureus* detected. The isolated *S. aureus* were screened for the presence of the clumping factor encoding gene (*clfA* gene) by PCR; all of the isolated 105 (100%, 105/105) *S. aureus* strains carried the *clfA* gene. The isolated *S. aureus* were molecular screened for the presence of the staphylococcal enterotoxin encoding genes (SEs); *Sea, Seb, Sec, Sed*, and *See* by multiplex PCR; 6 (5.7%, 6/105), 4 (3.8%, 4/105), and 3 (2.9%, 3/105) carried the, *Seb, See, Sed* genes respectively. The detection of 13 (12.4%, 13/105) enterotoxigenic *S. aureus* strain in the present investigation is considered a potential public health hazard which should be taken into consideration to find the possible strategies for prevention. The most frequent enterotoxin encoding genes detected were *seb* 6 (5.7%, 6/105), *see* 4 (3.8%, 4/105), and *sed* 3 (2.9%, 3/105) respectively. There was not found any *S. aureus* carried neither for *Sea* nor *Sec* genes.

Keywords: Cheese; enterotoxigenic S. aureus; clfA; Sea-See; milk.





Antibacterial Effect of Gold Nanoparticles against Corynebacterium

Pseudotuberculosis

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Corynebacterium pseudotuberculosis is the etiological agent of the chronic disease known as caseous lymphadenitis (CLA). The bacterium infects goats and sheep causing great economic losses worldwide annually. The objective of this study was to compare between the uses of gold nanoparticles (AuNPs) alone as antibacterial active agent against C. pseudotuberculosis bacteria and AuNPs – laser therapy. The bacterial growth rates were investigated by finding the minimum inhibitory concentration (MIC) of three different concentrations of AuNPs (50, 100 and 200 µg/ ml) as well as measuring the bacterial growth curves in the present and absent of laser. The mechanism of interaction between AuNPs and bacteria was analyzed through bacterial thin sections followed by transmission electron microscope (TEM) analysis. Confocal laser scanning microscope was used to evaluate the cytotoxic action of AuNPs on C. pseudotuberculosis. As a result, MIC of AuNPs against C. pseudotuberculosis was 200 µg/ ml while the MIC of AuNPs-laser enhanced to be 100 μ g/ ml. The laser light could enhance the antimicrobial activity of gold nanoparticles by at least one fold. So it could be concluded that the AuNPs combined with laser exposure could be used as an effective antibacterial material to inhibit the growth of C. pseudotuberculosis.

Keywords: Antibacterial agent; caseous lymphadenitis; *C. pseudotuberculosis*; gold nanoparticles; laser.





Demodectic mange caused by four *Demodex* species in different dog breeds in Egypt

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In Egypt, examination 175 dogs of different breeds (German shepherd, Boxer, Rottweiler, Saint Bernard and Doberman) during 2016 revealed that, 12% of dogs were suffering from demodectic mange of different species. The highest percentage of infection was in *German Shephered* (28.57%). *D. canis* showed the highest prevalence among examined dog breeds (71.4%) followed by *D. cornei* and *D. injai* (9.5% each) then the undefined species with 4.7%. For the first time the life cycle of *D. canis* among *Rottweiler* was studied and recorded. The histopathology changes were also studied. Treatment using special protocol was adopted which leads to complete cure of infected dog.

Keywords: *D. canis*; *D. cornei*; *D. injai*; life cycle; treatment and histopathology; unidentified species.





Anthelmintic effect of Artemisia herba-alba against *Heterakis* gallinarum infecting turkey poults

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The present study was conducted to investigate the anthelmintic effect of Artemisia herba alba extract on the turkey poults infested with invasive *Heterakis gallinae* eggs. Sixty, one-day-old, white male turkey poults were used. The birds were divided into 5 equal groups, 12 each. The first group was the negative control group (non-infected, nonmedicated). Birds in remaining groups (2, 3, 4 and 5) were infested with a single dose of 500 embryonated eggs of Heterakis gallinarum per bird one day after hatching. The second group was the positive control group (infected, non-medicated). The third group was treated with albendazole (20%) at 25 days post infestation (PI) in a dose of 5 mg/kg B. wt. in drinking water for 3 consecutive days. The fourth group was treated with aqueous extract of Artemisa herba-alba (AEA) at 25 days PI (400 mg/ kg. b. wt.) in drinking water for 3 consecutive days. The fifth group was treated with AEA at 25 days PI (800 mg/ kg. b. wt.) in drinking water for 3 consecutive days. AEA induced anthelmintic effect as it reduced egg shedding and worm burden in the infected birds, in a similar manner to that of albendazole. AEA produced significantly improved FCR and had no adverse effects on liver and kidney. It could be concluded that, AEA could be used for controlling heterakid infection as an alternative to standard anthelmintic drugs.

Keywords: Anthelmintic; artemisia herba-alba; turkey poults.





Sarcoptic donkey's mange: a growing problem in Upper Egypt and strategic controlling measures in infested animals and animal environment.

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The present study was conducted on 630 donkeys during the period from March 2014 to April 2015. These animals belong to different villages in Assiut Governorate, Upper Egypt. The age of these animals ranged from less than one year to above ten years old. Examination of diseased animals revealed that they suffered from itching, loss of patches of hair in different regions of the body with appearance of scales on the skin. The more common sites of infestation were the leg, base of the tail, neck and the head of animals. In this study skin scrapings were examined microscopically revealed that Sarcoptic Scabiei equi was the main identified mite from infested donkeys in Upper Egypt in percentage of 13.12%. Statistical analysis of some ecological and management parameters revealed that there is a significant relationship (p<0.05) between prevalence of mange mite infesting donkeys and season, age, housing management as well as regular or irregular using acaricides. Moreover, this study included using different methods of clinical therapeutic trails. The infested donkeys with Sarcoptic mites were classified into four groups. The first one received two doses of Ivermectin (Ivomec, Merial), 14 days apart at dose rate of 200µg/kg oral paste. The second group received two doses of doramectin (Dectomax, Pfizer, Egypt) a, 14 days apart at dose rate of $200\mu g/kg$ oral paste. The third group received two doses of Ivermectin (Ivomec, Merial), 14days apart at dose rate of 200µg/kg oral paste; Adjunct to this drug, Amitraz was applied to the surrounding environment (bedding material, wall, fomites, etc...) twice at a 14 days interval. All cases were isolated in a separate place during treatment period. The fourth group received two doses of doramectin (Dectomax, Pfizer, Egypt) a, 14 days apart at dose rate of $200 \mu g/kg$ oral paste, Adjunct to this drug, Amitraz was applied to the surrounding environment twice at a 14 days interval. We found that administration of ivermectin or doramectin adjunct with treatment of animal environment is the best protocol for eradication and prevention of mange mite from infested donkeys with mange mite and its environment.

Keyword: Amitraz; donkeys; Doramectin; environment; Ivermectin; mange.





Modern Assessment of Leishmaniasis in Balady Dogs in Egypt Mostafa M. Bashandy¹, Wahid M. Musa², Khalid A. El-Olemy³ and Mohammad A. Abo Shwreb⁴

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Canine leishmaniasis (CanL) is a severe zoonotic disease caused mostly by the Leishmania infantum, and transmits to dogs by the bite of Phlebotomus sand-fly. It is endemic in more than 70 countries worldwide, including the Mediterranean basin. Although some infected dogs present diverse clinical signs, others remain asymptomatic. The study aims to determine the prevalence of CanL infection in Balady dogs in some areas of Giza and Alexandria, Egypt, to assess associated hematological and some biochemical changes, and to evaluate the performance of four diagnostic procedures; parasitological examination, rapid immunochromatographic test, ELISA and PCR. Ninety nine Balady dogs, of different ages and of both sexes, were studied. There was not a significant difference in the infection rate regarding both age and sex. The commonly observed clinical signs were cutaneous. Within the hematological parameters, significant decreased hematocrit and hemoglobin concentration, leucocytosis and lymphocytosis, together with insignificant differences in erythrocyte, granulocyte, monocyte and platelet observed in CanL infected Biochemically, counts were dogs. significant hypoalbuminemia, hyperglobulinomia, increased ALT and AST activities as well as azotemia were also detected with CanL. Finally, the used diagnostic techniques showed a comparable performance.

Keywords: Dog; ELISA; hematology; Immunochromatography; Leishmania; PCR.





Endoscopic Examination of some Orodental Affections in Donkeys

(Equus asinus)

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Donkeys constitute an important part of livestock agriculture, producing milk, hid and draft power. This study aims to examine some orodental affections in donkeys by a flexible oral endoscopy. Routine oral examination was performed on 20 working donkeys at the surgery clinic, Faculty of Veterinary Medicine, Cairo University, Egypt. These animals aged 1-8 years and included 12 males and 8 females. Under general anesthesia, full visual, manual and endoscopic examinations were carried out for gingiva, teeth, hard palate, soft palate and tongue. The visual and manual examinations were carried out with the aid of a strong headlight, long handled equine dental mirror and mouth gag. Oral endoscopic examination was performed for detection of any abnormal lesion by using video endoscope, protected by a rigid metal sheath and recorded onto an analogue videotape. The results of the present study revealed that all of the presented donkeys suffered from single or mixed oral affections. The recorded oral affections included; dental tartar (90%), injuries of hard palate (66.7%), ulcerative gingivitis (85%), hard palate candidiasis (33.3%), per-incisor diastema (28.5%) and cheek teeth diastema (71.5%). All endoscopic findings of these affections were recorded. In conclusion, endoscopic examination is a helpful diagnostic tool for diagnosis of orodental affections, especially those of the caudal oral cavity of the donkeys.

Keywords: Dental caries; dental tartar; donkeys; hard palate; sharp teeth; stomatitis.





Chemical Castration versus Standard/Knife Surgical Castration in

Tom-cats: Clinical and Biochemical Study.

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For many years, surgical castration has been the only reliable and permanent method of contraception in small animals. Non-surgical methods for sterilization of male through intratesticular injection of a chemical substance as calcium chloride 20% were documented by world society for protection of animals (WSPA). The aim of the present study is to compare between standard surgical and chemical castration through intratesticular injection of calcium chloride (CaCl₂) 20% techniques in tom-cats through clinical, biochemical observations. The present study was conducted on 15 domestic shorthair tom-cats with age ranged from 9 months to 5 years and weight of 2.5-5 kg. The animals were distributed randomly into control group (GI) (Placebo group) containing 5animals and 2 main groups, 5 animals for each according to the used castration technique; surgical/knife technique group (GII) and CaCl₂ technique group (GIII). All animals were subjected to daily examination general health condition and the scrotal region was inspected. Complete blood picture (CBC), testosterone hormone and cortisol levels were estimated. The chemical castration showed more economic advantages than the surgical castration. Neither intra/post-operative complications were observed in chemical castration, unlike in the surgical method. CBC showed differences in WBC values in the surgical group than the chemical castration group. Blood cortisol levels were significantly different from day 1 to 28 in all castrated groups as compared with control. The effect of castration method on blood testosterone level was significantly decreased in all castrated groups as compared with control from Day 14 onwards. The chemical castration using intratesticular injection of calcium chloride 20% tincture solution was superior to the standard surgical technique as a safe and a cheap method especially for stray animals overpopulation control programs.

Keywords: Calcium chloride; castration; chemical; surgical; tom-cat.





Development of a new method for transfer of large equine embryos in Arabian Mares

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In the present study, there were 36 Arabian mares (7-24 years old) used as donors for embryos. The flush was carried out on day 10-11 post ovulation. Flushing of the uteri of 2 mares (died suddenly) was carried out after excision of the uterus. The recipient mares were (N=70) between 5-10 years old. The degree of synchronization was -4 to -6 days. The procedure was depending on flushing of the donor mares just after detection of embryonic sac using ultrasonography. Large pore AI catheters and external sheath of double guarded uterine swabs were used in the process of embryo transfer. A controllable manual pipette was used in the control process of loading, washing and transfer. This method overcomes the problem of burst of large embryos. The current study characterized by high recovery rate (97.2%) and high pregnancy rate (71.4%). The results also showed that higher pregnancy rates obtained with recipient mares on day 4 post ovulation and lower pregnancy rates in recipient mares on day 6 post ovulation. In conclusion, this study demonstrated that: there was a possibility of embryo transfer on day 10-11 post ovulation i.e. after embryo detection with ultrasound scanning. This method permits flushing of mare's uterus after death on 10-11 days of pregnancy for exploitation trail of the donor mare. Furthermore, concerning mares with a history of low embryo recovery flushing did not take place until embryo detected with ultrasound so as to safe flushing media and time numbers of flushes.

Keywords: Donor; embryo transfer; equine; large embryos; mare; synchrony.





Application of nanotechnology to differentiate between healthy and cancer cells through SEM detection of folate receptor

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Unlike the healthy cells, folate receptors (FR) are over-expressed on a wide range of tumor cells, including breast cancer cells. Targeting of cancer cells using specific surface molecules such as folate receptors may be one of the strategies used to kill cancer cells without hurting the neighing healthy cells. The aim of the current study was to try a method of SEM detecting FR in a murine breast cancer cell model (4T1 cells) using secondary antibody conjugated to gold or gold-coated magnetite nanoparticles. The 4T1 cells were suspended in RPMI medium with FR antibody and incubated with secondary antibody for fluorescence microscopy. The cells were cultured on 30mm Thermanox coverslips for 18 hours, labelled with FR antibody then incubated with secondary antibody conjugated to gold or gold-coated magnetite nanoparticles and processed to scanning electron microscopy (SEM) analysis. The fluorescence microscopy study showed strong punctate FR expression on 4T1 cell membrane. With SEM, the labelling with gold or gold-coated magnetite conjugates showed a similar pattern. Specific labelling occurred in nanoparticle clusters, which are clearly visualized in backscattered electron images. The 4T1 tumor cell model may be useful for the development of FRtargeted tumor therapy using gold-coated magnetite nanoparticles. The SEM detection of antigen-antibody complex using gold or gold-coated magnetite conjugates could be a challenge in the field of histology.

Keywords: Cancer cells; nanotechnology; SEM.