

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Table of contents

	Page
Dr. Iman Shaheed welcome message	1
Dr .Sohair Sokar welcome message	2
Final agenda	3
Scientific Board	6
Abstracts of Oral presentations	7
Poster Abstracts	31
Full manuscripts	46
Speakers biographies	204

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Welcome Message

Dear colleague,

It's my great honor and pleasure to invite you to attend the 1st International Conference of Pathology, Department, Faculty of Veterinary Medicine, and Cairo University which will be held In April 25-27 2013. The conference objectives are to promote international exchange and progress in the field of Veterinary Pathology, and to enhance international collaborations worldwide.

The conference will feature internationally recognized speakers, oral and poster sessions. The scientific programs will cover topics of almost all aspects of Veterinary Pathology, including fundamental researches in Pathology and Clinical pathology, Tumors, Infectious Diseases and Toxicopathology. Participants will benefit from the meeting and interacting with the specialists for refreshing knowledge base and skills, and this in addition to having the opportunities for establishing long-term international collaborations. I ensure you that conferences are internally influential events.

The event will be held in Cairo "The City of a Thousand Minarets" for its preponderance of Islamic architecture, Cairo has long been a center of the region's political and cultural life. Cairo was founded by the Fatimid dynasty in the 10th Century, but the land composing the present-day city was the site of national capitals whose remnants remain visible in parts of Old Cairo. Cairo is also associated with Ancient Egypt due to its proximity to the ancient cities of Memphis, Giza and Fustat which are nearby to the Great Sphinx and the pyramids of Giza.

On behalf of the organizing committees, I cordially welcome you to the exciting and stimulating events, and wish you all enjoyable time.

Iman Shaheed , Ph.D

Chairman of Conference and Head of Pathology Department

Faculty of Veterinary Medicine,

Cairo University, Giza , Egypt

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Welcome Message

Dear Colleagues

On behalf of the members of the Pathology Department, I welcome to our guest, and thank you for accepting our invitation and participation in First International Conference of Pathology Department, Faculty of Veterinary Medicine, Cairo University. I would also like to extend my sincere thanks to Prof. D. Iman Bakr Shaheed , head of Pathology Department , and the Chairman of the Conference on the work done until this conference appears in its supervisory. In the same context, I especially thank our guest from different countries and universities who endured the hardship of travel to our beloved Egypt; to communicate with each other's. This conference comes within the framework of the desire to achieve scientific communication between the departments of pathology locally and globally, and to share expertise, and access to the latest scientific research in the field of pathology and modern techniques to diagnose the disease, or other things that contribute to the achievement of scientific additions to the field of pathology.

Welcome again and we hope that the conference will be a step in achieving a permanent scientific cooperation between us, in terms of participation in scientific research, or arbitration, or the work of joint research projects. Finally, I pray to God - the Almighty -that we spend all the days of this conference in a scientific atmosphere, away from what is going around us internally or externally as tension and anxiety disorder. May God help us all to the good?

Prof. Dr . Sohair Sokkar PH.D

Honorable Chairman

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Conference Program

25 April 2013

9.30-10.30	Registration
10.30-11.30	Opening ceremony
11.30- 12	Coffee Break
	1 st Session
Session Presidents	Prof. Dr. Mohamed Ibrahim Dessouky Prof. Dr. Amira Hassan
12 – 12.45	Prof. Dr. Mary Christopher Global Alliances in Veterinary Pathology Teaching, Diagnostics, and Research.
	2 nd Session
Session Presidents	Prof. Dr. Sohair Sokkar Prof. Dr. Mohamed Osama El Shazley
12.45- 13.15	Prof. Dr. Mahmoud Abdel Salam Attia Chronic inflammation and neoplasia.
13.15-13.45	Dr. Mahir AG. Kubba Pathological investigation in the death of a captive ostrich (Struthio camelus) with a special reference to endocardiosis
13.45-14.45	Lunch
	3 rd Session
Session Presidents	Prof. Dr. Nabiha Ramdan Prof. Dr. Asharaf Shamaa
14.45 – 15.15	Dr. Marco Patruno Regenerative strategies applied to Veterinary Medicine

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Conference Program

15.15- 15.45	<u>Prof. Dr. Yesari Eroksuz</u> Pyrrolizidine Alkaloidosis: Pathogenesis and Pathological Features
26 April 2013	
9.30-10.00	Registration
	1st Session
Session Presidents	Prof. Dr. Mamdouh Affify Prof. Dr . Hala El Miniway
10-10.30	<u>Dr. Ausama, A. Yousif</u> Rise of the Egyptian Biosheild
10.30- 10.45	<u>Prof. Dr. Yesari Eroksuz</u> Acute and Subacute Fetal Aflatoxicosis in Two Dogs
10.45 – 11.00	<u>Dr. Kamal Zidan</u> Abscesses in dromedary camels, sheep and goats : etiology and pathology
11.11.15	<u>Prof. Dr. Hatice Eroksuz</u> Persistent Truncus Arteriosus, Left Ventricular Hypoplasia and Coronary Artery Malformations in a Calf
11.15- 13.00	Coffee Break and Pray
	2nd Session
Session Presidents	Prof. Dr Rawhia Doghaim Prof. Dr. Magdi Mohamed El Mahdy
13.00 -13.15	<u>Dr. Sherein Said Abdelgayed</u> Evaluation of avian influenza vaccines on commercial male layer chicks

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Conference Program

13.15-13.30	<u>Dr. Mohamed Abdel Rahman Bosila</u> Further Pathological studies on Ornithobacterium Rhinotracheal infection in chickens with trial for vaccine preparation.
13.30 – 13.45	<u>Dr. Mohamed Sayed Ahmed</u> Clinical and pathological Characterization of Rabbit Haemorrhagic Disease in the Sultanate of Oman
13.45-14.00	<u>Prof. Dr. Sayed R. Al-Attar</u> Pathological Evidence for a Fatal Nervous Disease in Grouper Fish in Libya
14.00-14.15	<u>Dr. Mouchira M. Mohi El-Din</u> Patho-physiological Studies On the Effects of Confidor (Imidacloprid) on Albino Rats
14.15- 14.30	<u>Dr. Khadra Soliman</u> Pathology of Metalaxyl Fungicide on Rabbit Kidney and Urinary Bladder.
14.30- 14.45	<u>Randa A. Hassan</u> Toxic Effects Of Subacute And Subchronic Intoxication Of Profenofos In Albino Rats
14.45- 15.00	<u>Dr. Mahir AG. Kubba</u> Ceruminous Gland Adenocarcinoma in a Cat
15.00- 16.00	Lunch
	27 April 2013
9.30- 10.00	Registration
	1st Session
Session Presidents	Prof. Dr . Moustafa Bashandy Prof. Dr. Alaa Raffat
10.00- 10.45	<u>Prof. Dr. Mary Christopher</u> Exfoliative Cytology: Principles and Practice

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Conference Program

2 nd session	
Session President	Prof. Dr. Iman Shaheed
10.45- 11.15	Dr. Mohamed Abdel Razik New trends in teaching practical veterinary histology and pathology
11.15- 11.45	Coffee Break
11.45- 12.15	Assessing of best poster
12.15- 12.45	Closure Ceremony

Scientific Board

Abdel Azim Khalaf	Prof. of Toxicology, Cairo University
Alaam Nafady	Prof. of pathology, Assuit University
Iman B. Shaheed	Prof. of pathology, Cairo University
Mohamed Osama El Shazley	Prof. of pathology, Cairo University
Mahir Kubba	Assistant professor of pathology, Tripoli University
Mamdouh Affify	Prof. of pathology, Cairo University
Manal Affify	Prof. of poultry disease, Cairo University
Mohamed El Sayed	Prof. of Toxicology, Cairo University
Salah Ali	Prof. of pathology, Suez Canal University
Sary Khalil	Prof. of pathology, Assuit University
Sayed R. Al-Attar	Prof. of pathology, Zagzag University
Sohair Sokkar	Prof. of pathology, Cairo University
Rawhia Doghaim	Prof. of pathology, Cairo University

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Oral Presentations

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Thursday , 25/4/2013

1st Session

12.00- 12.45

Global Alliances in Veterinary Pathology Teaching,
Diagnostics, and Research

Global Alliances in Veterinary Pathology Teaching, Diagnostics, and Research

Mary M. Christopher, DVM, PhD, Dipl ACVP, Dipl ECVCP

University of California–Davis

Global alliances in veterinary pathology are important to focus resources on important and relevant animal diseases; to bring together diverse perspectives and optimal expertise to solve critical problems; to stimulate new ideas and advance knowledge; to engage students and train the next generation of pathologists; and to benchmark our own institutions and efforts. Effective collaborations require leadership, trust, and clear strategies for ensuring open communication, shared responsibilities, and shared credit. I will focus on how international collaborations enhance capacity, competence, and community in veterinary pathology. Research collaborations and networks greatly enhance our **capacity** for addressing regional and global animal (and human) health problems, and increase the impact of our research. International collaborations enhance **competence** through shared resources and models of specialized training and standards for veterinary pathologists. Teaching collaborations promote curricular innovation to enhance critical thinking and problem-solving, and improve global competitiveness through educational opportunities. International alliances develop the veterinary pathology **community** through

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



national and international organizations that support education, research, and diagnostic training and facilitate cultural awareness.

2nd Session

12.45- 13.15

Chronic inflammation and neoplasia

Chronic inflammation and neoplasia.

Prof. Dr. M. A. ATTIA (BVMsc, DVP, PhD)

Expert pathologist and independent consultant, Former scientific director and director of pathology and clinical pathology at pharmaceutical companies and CROs in the Netherlands and France.

It is generally accepted that chronic inflammation-triggered by toxins, microbes (including vaccines) or autoimmune reactions, plays a major role as a tumor promoter.

Inflammation may become chronic either because an inflammatory stimulus persists or because of dysregulation in the control mechanism that normally turn the process off. Many of the cell types, cytokines and systems (e.g., leukocyte migration, dilatation of the local vasculature and angiogenesis) involved in inflammation are also found in a variety of tumors. Among pro-inflammatory gene products involved in such interactions are tumour necrosis factor (TNF)-alpha, interleukin (IL)-6 and vascular endothelial growth factors (VEGFs), whose expression is mainly regulated by the transcription nuclear factor (NF)- kappaB (transcription activator) , which induced by TNF-alpha. Tumor cells produce various cytokines and chemokines that attract leukocytes, which in turn produce cytokines and chemokines that stimulate further tumor cell proliferation; the inflammatory tumor microenvironment is characterized by the presence of host leucocytes both in the stroma and around tumor.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



The practical and experimental examples for the relationship between chronic inflammation and neoplasia are Asbestos and MMVF (Man Made Vitrous Fibers)-induced mesotheliomas and Vaccine associated sarcoma. The findings of these experimental works will be discussed in detail.

13.15-13.45

Pathological investigation in the death of a captive ostrich (*Struthio camelus*) with a special reference to endocardiosis

Pathological investigation in the death of a captive ostrich (*Struthio camelus*) with a special reference to endocardiosis

Mahir AG. Kubba and Seham A. Al-azreg

Department of Pathology, Faculty of Veterinary Medicine, University of Tripoli

A seven years old blue-necked male ostrich was found dead after few days of illness. The animal was kept in an open yard of 25 square meters along with three other females. They were given concentrate-rich ration with free access to green leaves and water. Autopsy revealed cardiac enlargement due to left ventricular hypertrophy and right ventricular dilatation. The left aterio-ventricular valves were irregularly thickened and contracted. The lungs were engorged with blood and the liver had nutmeg appearance. The small intestine showed segmental subserosal petechial hemorrhages. Histological examination revealed myxomatous degeneration of the valves, pulmonary congestion and edema, congestion of periacinar hepatic zone and fatty degeneration of outer zones, renal glomerular sclerosis with arteriosclerosis. The affected parts of the small intestine showed villous atrophy with lacteal distention and severe sub-mucosal venular dilatation. The submucosal arterioles showed luminal narrowing and wall degeneration.

Endocardiosis is a well-known cardiac disease affecting certain breeds of dogs. It is basically a myxomatous degeneration of the valves which mainly involve the left aterio-ventricular valves leading to mitral insufficiency. Other cardiac valves are also affected but with less severity and

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



frequency. The affected valve is shortened and thickened, opaque white with smooth surface and no signs of inflammation. Microscopically, the most prominent features of endocardiosis is thickening of the valvular spongiosa and degeneration of the fibrosa. The thickening of the former is due to proliferation of loose fibroblastic tissue rich with proteoglycans, hyaluronic acid and chondroitin sulfate (Jones et al, 1997, Jubb et al. 2007, Merk, 2011, Fox PR, J. Vet. Card. 2012). The cause of endocardiosis is not known but is likely a genetically influenced degeneration of connective tissue. Though infrequent, valvular abnormalities suggestive of endocardiosis have also been reported in cats (Sisson, 1978), horses (Reef et al, 1998), pigs (Castagnaro et al. 1997), Gambian rats (Kerstin et al. 2010), rhesus monkeys (Schmidt, 1970) and in aviary birds (Schmidt et al. 2008). This condition bears some resemblance to a condition in human referred to as mitral valve prolapse (Jones et al. 1997). The current paper describes and discusses a condition in a captive ostrich which correspond well with endocardiosis in other animals.

3rd Session

14.45-15.15

Regenerative strategies applied to Veterinary Medicine.

Regenerative strategies applied to Veterinary Medicine.

Patruno M. *, Maccatrazzo L. *, Perazzi A. **, Iacopetti I. **, Spaas J. *, Martinello T. ***

* Department of Comparative Biomedicine and Food Science, University of Padova, Italy

** Department of Animal Medicine, Production and Health, University of Padova, Italy

*** Global Stem cell Technology, Geeneindestraat 1, Meldert-Lummen, Belgium

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Regenerative veterinary medicine is recently using tissue engineering techniques and cell-based therapies for the treatment of acute and chronic pathologies. Our group studies frequently occurring conditions as tendon's traumatic rupture and tendinopathies attempting to improve the "intrinsic" tendon healing. For example, injection of mesenchymal stromal cells (MSC) into the core of a damaged tendon aims to take maximal advantage of reparative mechanisms that occur naturally in the animal body, as fibroblast migration to the site of defect. In this report the most significant results and future strategies of our research are summarized, emphasizing the fact that adult stem cells obtained from different sources are safe and have the potential to enhance functional recovery in different equine injuries. However, it will be necessary to increase the number of clinical and experimental cases in a long-term follow-up period for evaluating re-injury percentages and analyze the histological and molecular parameters of the healed tissues.

15.15- 15.45

Pyrrolizidine Alkaloidosis: Pathogenesis and Pathological Features

Pyrrolizidine Alkaloidosis: Pathogenesis and Pathological Features

Yesari Eröksüz, Hatice Eroksuz, Aydın Çevik, Burak Karabulut , Şevket Soylu

Department of Pathology, Faculty of Veterinary Medicine, Firat University, 23200,
Elazig, TURKEY

Pyrrolizidine alkaloids (PAs) are found in many plants genera including Heliotropium, Senecio, Amsinkia, Cynoglossum Echium and Trichodesma. Ingested alkaloids are converted pyrrolic esters which are alkylating agents, which react with cytosolic and nuclear proteins. There are 3 common pathological expression of PA poisoning including: 1.Acute periacinar zonal necrosis, occurs after ingestion of large amounts (rare in natural outbreak). 2. Regenerative

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



nodule formation, megalocytosis, veno-occlusion (rare) 3.Firm atrophic livers without nodular regeneration (prolonged exposure)

To establish plant-lesion relation is extremely important in differential diagnosis of other alkylating agents including aflatoxicosis and nitrosamin poisoning.

Friday , 26/4/2013

1st Session

10.00- 10.30

Rise of the Egyptian Biosheild

Rise of the Egyptian Biosheild

Ausama A Yousif, Ph. D.

Associate professor of Virology, Faculty of Veterinary Medicine, Cairo University

In the years leading to the Egyptian revolution national economic assets depending on animals, birds, and fish suffered devastating consequences due to the introduction of transboundary pathogens. The Egyptian public health was not spared the force of the impact. The national budget continues to pay the cost lacking an effective Biosheild strategy. Rise of the Egyptian Biosheild requires analysis of biothreat sources, survey of current and prospect technologies, local manufacture of vaccines and diagnostics, redefining of national biological borders, implementation of preemptive vaccine-based control strategies, issuing of effective legislation to coordinate Biosheild activities, reformatting of the modus operandi of national response sectors, and raising public awareness of the nature of the biological threat. The focus of this presentation will be raising awareness of the nature of the biothreat, definition of the national

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



biological borders, the available and future technology tools that can be used within the Egyptian Biosheild, and a proposed unified discovery oriented research system. Key legislative and operational requirements will be discussed.

10.30- 10.45

Acute and Subacute Fetal Aflatoxicosis in Two Dogs

Yesari Eroksuz¹, Ertugrul Kaya², Mustafa İssi³, Ersoy Baydar³, Aydın Çevik¹, Hatice Eroksuz¹

¹Department of Pathology, Faculty of Veterinary Medicine, Firat University, 23200 Elazig, TURKEY

²Department of Pharmacology, Faculty of Medicine, Duzce University, 81620, Duzce, TURKEY

³Department of Internal Medicine, Faculty of Veterinary Medicine, Firat University, 23200 Elazig, TURKEY

Acute and subacute aflatoxicosis due to moldy bread was reported in two dogs as separate premises. The both dogs had anorexia, deep depression, however the first dog died in 24 hours of the onset clinical signs including melena, lateral recumbency and epistaxis. Severe diffuse icterus, severe diffuse hemorrhage in the intestines, enhanced lobular pattern of the liver, mesenteric arterial thrombosis, diffuse, severe gastric, splenic and pancreatic edema were the main findings detected. Serum chemistry abnormalities, included marked elevation of alanine aminotransferase (474 IU/L), alkaline phosphatase (463 IU/L) and blood urea (192 mg/dl), indicated the hepatic and renal failure. Microscopic liver findings were characterized by submassive to periportal necrosis, diffuse moderate fatty change, early regenerative nodule formation, periportal congestion, bile pigment deposits, binucleated hepatocytes and mild karyomegaly. In the second case; the clinical signs and pathologic findings were much milder, and diffuse, moderate fatty change, fibrosis and bile duct proliferation were the main

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



microscopic findings detected. Mushroom poisoning in both cases was excluded by examining the livers, kidney and urine samples by HPLC method in regard to alpha, beta and gamma amanitin.

Exposure to aflatoxin was confirmed by determining the levels of aflatoxin (B1, B2, G1, G2) or their metabolite (M1) in the bread, liver, kidney and urine samples by HPLC method.

10.45 – 11.00

**Abscesses in dromedary camels, sheep and goats :
etiology and pathology**

Zidan, K.H¹; Mazloum, K.²; Saran, M.A.²; and Hatem, M.E.³

¹ Al-riyadh diagnostic veterinary laboratory, ² National Agriculture& animal Resources Research Center, Ministry of Agriculture, Al-Riyadh, Kingdom of Saudi Arabia 11195.

³ Fac. of Vet. Medicine, Cairo Univ., Egypt.

Abscesses of farm animals cost the producers tremendous losses. Forty five samples collected from dromedary camels in addition to two hundred and fifty samples collected from sheep and goats from Al-Riyadh, Kingdom of Saudi Arabia during 2012. Bacteriological examination of the samples revealed that the main causes of camel abscesses were *Corynebacterium* sp. (39%) and *Staphylococcus aureus aureus* (17%). Streptococci and rhodococcus were also isolated from few cases, while no bacterial growth was seen in 29% of the collected samples. Four subcutaneous dermoid cysts (8.8%) were also observed and described during the investigation that filled with hair and brownish watery fluid. On the other hand the main causes of abscessation in sheep and goats were *Staphylococcus aureus anaerobius* (55%) (cause of Morel's disease), *Corynebacterium pseudotuberculosis* (24.5%) (cause of caseous lymphadenitis CLA) and *Staphylococcus aureus aureus* (15%). The histopathological picture of camel lymph nodes (LNs) infected with corynebacterium was quite different from that appeared in CLA in sheep and goats.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



The current study showed that the infection of dromedary camels with abscesses is comparatively little and self limiting; unlike that in sheep and goats. The disease in sheep and goats can be controlled by avoiding the predisposing factors and using a mixed autogenous vaccine from local isolates.

11.00 – 11.15

Persistent Truncus Arteriosus, Left Ventricular Hypoplasia and Coronary Artery Malformations in a Calf

Persistent Truncus Arteriosus , Left Ventricular Hypoplasia and Coronary Artery Malformations in a Calf

Hatice Eroksuz¹, Sadık Yılmaz², Yesari Eröksüz¹, Aydın Çevik¹, Sevket Soylu¹, Burak Karabulut¹

¹Department of Pathology, Faculty of Veterinary Medicine, Firat University, 23200, Elazig,
TURKEY

²Department of Anatomy, Faculty of Veterinary Medicine, Firat University, 23200, Elazig,
TURKEY

Morphologic features of persistent truncus arteriosus with left ventricular hypoplasia, patent foramen ovale and intact ventricular septum were described in a 3-day-old Holstein calf. The left coronary artery was originated from the brachiocephalic (arterial) trunk. There was apparently no right coronary artery. There was also a fistular connection in the lateral margin of left ventricle communicating with the left atrium at the level of pulmonary vein. Microscopically, there was medial thickening of the wall of arteries and arterioles in the lungs and in the left ventricle. There was moderate diffuse endocardial fibrosis in the left ventricle. There was moderate edema and

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



chronic congestion and features of aspiration pneumonia characterized by lympho-plasmacytic infiltration, fibrinous exudation, and free fat vacuoles in the lungs.

As a result, the present case is unusual in many respects and combination of these factors might contribute the early death

2nd Session

13. -13.15

Evaluation of avian influenza vaccines on commercial male layer chicks

Evaluation of avian influenza vaccines on commercial male layer chicks

M.M.Amer¹, Sherein, S.abdelgayed², Abeer, A. Abd El-Baky ³ and El- A Akasha⁴

¹Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, Egypt

²Department of pathology, Faculty of Veterinary Medicine, Cairo University, Egypt

³Department of Clinical Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt

⁴Veterinary Clinic, Faculty of Veterinary Medicine Beni-suef University, Egypt

Avian influenza (AI) virus was recognized as a highly lethal, systemic disease resulting in high economic losses in poultry industry worldwide. On rare occasions, AI viruses have exhibited interspecies transmissibility to human. Inactivated avian influenza (AI) virus vaccines have been used in a variety of avian species and their effectiveness in preventing clinical signs and mortality is well documented. In the present study, a total of 90 one day old commercial male layer chicks were used for evaluating monovalent (H5N1) and bivalent (H5N1 + ND) inactivated avian influenza vaccines. All chicks were reared on floor housed system and were fed

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



ad libitum on a balanced commercial ration. The chicks were divided equally into 3 groups as follow; A, B and C each group contains 30 chicks. Chicks of groups A and B were vaccinated with monovalent (H5N1) and bivalent (H5N1 + ND) inactivated AI vaccine by subcutaneous route (s.c) at 9th day, respectively. Chicks of groups C, acted as a control non vaccinated group. This study continued for 5 weeks through which collection of samples was performed weekly. Feed intake, body weight gain, feed conversion rate (FCR) and organ/body weight ratio were carried. Immunological evaluation was carried using haemagglutination inhibition antibody (HI) test against avian influenza virus (AIV). Total erythrocyte and leukocyte counts, packed cell volume (PCV %), hemoglobin concentration and differential leukocytic count were performed to evaluate the clinico-hematological effect of AI vaccines. Serum total proteins, albumin, globulins, uric acid and creatinine concentrations were done. Also, hepatic enzymes activities and blood glucose level were carried to evaluate clinico-biochemical effect of AI vaccines. Examination of bursa, thymus, liver and spleen was carried to evaluate histopathological effect of AI vaccines. Results of feed intake, body weight gain, FCR, organ/body weight ratio and HI antibody titers against avian influenza virus H5N1 revealed, the monovalent (H5N1) vaccinated group has higher values than bivalent (H5N1+ ND) vaccinated group. The clinicopathological changes revealed the presence of significant leukocytosis and hyperproteinemia in both vaccinated groups.

Bursa, thymus and spleen sections of control were apparently normal at all intervals. Bursal section of 2 weeks vaccinated chicks showed inter follicular congestion in AI vaccine and perifollicular congestion In AI+ND. AI vaccine showed interfollicular fibrosis at 3 weeks. In AI + ND group at 4 weeks, perifollicular edema with follicular atrophy were seen while marked follicular atrophy was detected at 5 weeks. Thymus section of AI and AI + ND vaccinated groups at 3 weeks showed congested medulla as well as necrosed medulla in 5 weeks. AI gr at 4 weeks showed slightly necrosed medulla and necrosis in cortex at 5 weeks. Spleen section of AI vaccinated chickens group showed severs congestion with vasculitis at 4 week and slightly

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



depleted follicles at 5 weeks. Severe congestion in follicles at 4 weeks and necrosed follicles at 5 weeks were detected in AI +ND vaccinated group.

13.15 – 13.30

Further Pathological studies on Ornithobacterium Rhinotracheal infection in chickens with trial for vaccine preparation.

Further Pathological studies on Ornithobacterium Rhinotracheal infection in chickens with trial for vaccine preparation.

Mohamed Abdel Rhaman Bosila², Nabiha Ramdan Hassan¹, Iman B. Shaheed¹, M.A. Kutkat², K.M Mahgoub².

¹Department of Pathology, Faculty of Veterinary medicine, Cairo University, Egypt

²National Research Center, Giza

This study was conducted to investigate the pathogenicity of ORT and trials to testing the effect of water based Vaccine. A number of 160, one day old birds were divided into four isolated groups, 40 Birds each. The first group was kept as uninfected control group. The second group was vaccinated with 0.5 ml subcutaneous injection of inactivated whole bacterial cells culture water base vaccine at 14th day of age. The third group was aerosolized at 42nd day of age by field isolate of *Ornithobacterium rhinotracheale* using 100 ml peptone water containing 3.8×10^8 CFU/ml of *Ornithobacterium rhinotracheale*, using a commercial paint sprayer particle (size ≥ 50 Um). The fourth group was vaccinated at the 14th day of age, then at 42th day of age were challenged by field isolate of ORT, Five birds were slaughtered from each group at the 14th, 21st, 28th, 35th, 42nd, 49th and 56th day of age for bacteriological, serological and histopathological examination with light and electron microscope. Tissue specimens from nasal cavity, trachea, lungs, air sacs, proventriculus, gizzard, intestine, liver, kidneys, heart, brain, spleen, thymus and bursa were taken for the microbiological and histopathological examination. The clinical signs revealed respiratory manifestation with decrease in body weight in the 3rd group. The

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



microscopic examination of different organs of the 3rd group revealed congestion and necrosis of respiratory epithelium of nasal cavity and trachea. There were bronchitis, pneumonia and airsacculitis. Necrosis and hydropic degeneration were detected in liver and kidneys while the heart showed congestion and Zenker's necrosis. These results were not obvious in the 4th group.

13.30 – 13.45

Clinical and pathological Characterization of Rabbit Haemorrhagic Disease in the Sultanate of Oman

Clinical and pathological Characterization of Rabbit Haemorrhagic Disease in the Sultanate of Oman

*Mohamed S. Ahmed.^{1,2)}, Body M.¹⁾, AL-Rawahi A. H.¹⁾, Al-Mawaly, M.¹⁾ and Al-Habsy, S.¹⁾

¹Department of Pathology, Faculty of Veterinary Medicine, Kafr El-Sheikh University, Kafr El-Sheikh, 33516, Egypt.

²Veterinary Research Centre, Ministry of Agriculture and Fisheries, A'seeb. P.O.Box:50-Postal code: 121, Sultanate of Oman.

Rabbit hemorrhagic disease (RHD) is rapidly fatal viral disease of adult rabbits caused by a rabbit hemorrhagic disease virus (RHDV), a member of the genus *Lagovirus* of family Caliciviridae. Onset of the disease was sudden with 100% morbidity and mortality rate in the adult rabbits only in the farms of the area of study in Oman. The provisional diagnosis was reached by history, clinical signs, postmortem and histopathological findings and experimental inoculation in adult and juvenile *albino* laboratory rabbits. Adult rabbits inoculated with liver homogenate exhibited typical clinical pattern of the disease (incubation period, and percent

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



mortality), postmortem and microscopical lesions; while young rabbits did not develop the disease. Postmortem picture of experimentally infected rabbits was similar with little variation in severity of lesions to the rabbits with natural infection. The most prominent lesions found at necropsy were petechial haemorrhages in almost all organs. Microscopically, liver multifocal necrosis, pulmonary haemorrhages, lymphoid depletion and haemorrhages in the lymphoid tissues and the sub-epicardial haemorrhages in the heart were the most noticed changes.

Allantoic fluids harvested from inoculated embryonated eggs did not show any haemagglutination activity. The virus could not grow on embryonated chicken eggs. As far as could be ascertained, this is the first document on RHD from the Sultanate of Oman.

13.45-14.00

Pathological Evidence for a Fatal Nervous Disease in Grouper Fish in Libya

Pathological Evidence for a Fatal Nervous Disease in Grouper Fish in Libya

¹Sayed R. Al-Attar, ²Mahir Kubba, and ³Jameela T. Rizgalla

1-Department of Pathology , Faculty of Vet. Med. Zagazig University,Egypt.

2- Department of Pathology , Faculty of Vet. Med. Tripoli University. Libya.

3-Department of Aquiculture , Faculty of Agriculture, Tripoli University, Libya

Huge Grouper fish mortalities at the east Libyan cost in 2011 were intensively investigated. Clinical, gross and histopathologic examinations were carried out in addition to bacterial isolation and parasitic detection. Microscopic tissue examination revealed different tissue abnormalities in different organs. Of peculiar importance were those in the brain and eyes.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



The brain tissue showed congestion and edema in Virchaw Robin space with perivascular cuffing, multiple stroma vacuolation (malacia), neuronal degeneration, vacuolation and necrosis, associated with gliosis and lymphocytic aggregations. The eyes were congested and had hemorrhages and telangiectasis in the sclera and retina, lens degeneration and calcification along with corneal ulcers and hemorrhages. Such tissue changes were close to those reported by many authors to be associated with viral nervous necrosis in different kinds of fish including Groupers. Our results are also in comparable with that of institute of aquiculture, University of Stirling, Scotland. Though unconfirmed locally, adopting such etiological factor in causing this periodic massive mortalities would be a worth consideration.

14.00-14.15

Patho-physiological Studies On the Effects of Confidor (Imidacloprid) on Albino Rats

Patho-physiological Studies On the Effects of Confidor (Imidacloprid) on Albino Rats

^{1*}Mouchira M. Mohi El-Din and ^{2*}Rana A. Ali Ahmed

¹Pathology and Clinical Pathology Department, Faculty of Veterinary Medicine & ²Zoo Department, Faculty of Science, *South Valley University, Qena.

This experiment was conducted to study the toxic effect of confidor on rats. Thirty two adult female rats, at aged 2- 3 months, weight 150- 200g, were divided into four groups (n= 8 rat). Groups 1, 2 and 3 were orally received 45 mg/ kg of body weight confidor at a dose 1/10 of LD₅₀ every two days for 3, 6 and 9 times respectively. The group (4) was served as control. All administrated rats were sacrificed after the last dose of treatment (6, 12 and 18 day post-treatment) respectively. Blood samples were collected for hematological and biochemical parameters. Specimens from liver, kidneys and spleen were collected for histopathological

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



examination. The hematological results revealed a significant decreased in total RBCs counts, Hb amount and HCT percentage after high doses of confidor. The biochemical findings, a significant increase in glucose, total proteins, uric acid and creatinine, while cholesterol was elucidated significant decreased in high doses of confidor. The serum ALT, AST showed significant decreased in the rats which received confidor with different doses. Histopathology, showed degenerative changes and necrosis in the liver and kidneys, while depletion in lymphoid cells with hemorrhage in the spleen in different doses of the insecticide. It could be concluded that confidor with different doses had a toxic effects on visceral organs besides impairment of liver and kidneys functions.

14.15-14.30

Pathology of Metalaxyl Fungicide on Rabbit

Kidney and Urinary Bladder

Pathology of Metalaxyl Fungicide on Rabbit

Kidney and Urinary Bladder

Khadra, Soliman*; Sawsan, Jallah ; Afaf A El Ghawas* and Rawhia, E. Doghaim*****

*Department of Pathology, Animal Health Research Institute, Dokki, Giza

**Department of Pathology, Faculty of Medicine, King Abd Al Aziz University

*** Department of Pathology, Faculty of Veterinary Medicine, Cairo University

Metalaxyl sprayed ration was daily offered containing 0.05 mg/kg to young weaned New Zealand rabbits to study the toxic effect of such low dose for 24 successive weeks. Clinical pathology was done to give a mirror image for kidney functions. Gross examination, histopathology as well as transmission electron microscopic examination was performed. Marked renal failure was represented by significant rise of serum creatinin and blood urea nitrogen .In addition, there was a remarkable decrease in serum total proteins as well as albumin

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



in a dose time/related sequence. Gross examination revealed multiple deep depressed foci on the outer surface of kidneys with reduction of cortex. Marked thickened wall of urinary bladder was not uncommon. Histopathologically, glomerulonephritis accompanied by interstitial nephritis were recorded in an increasing intensity among rabbits examined after periods beginning from the 2nd weeks up to the end of experimental duration that extended until the 24th week. Hyperplastic papillomatous folds of bladder were thrown into the lumen and covered by stratified transitional epithelium could be detected.

Glomerular EM image after 12 weeks of experiment revealed mesangial cell proliferation accompanied by increased amount of mesangial matrix .Electron dense deposits were distributed inside such matrix. Proximal convoluted tubules EM image showed highly increased microvilli, many free ribosomes at the apical part of its cytoplasm as well as highly folded plasma membrane of tubular epithelial cells. Mitochondrial swelling was also observed. The most prominent changes observed after 24 weeks consisted of wrinkling of capillary basement membrane, fusion of podocyte's foot processes, and extensive proliferation of mesangial cells. Increased mesangial matrix was also detected in an extensive manner with electron dense deposits through it.

14.30- 14.45

Toxic Effects Of Subacute And Subchronic
Intoxication Of Profenofos In Albino Rats

Toxic Effects Of Subacute And Subchronic Intoxication Of Profenofos In Albino Rats

Rania Abd El-Al A. H. *; Nariman A. Rahmy**; Randa A. Hassan** and El-Araby E. K. M. ***

*Pharmacological Dept- National Organization For Drug, Control and Research (NODCAR)

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



** Pathology Dept-Animal Health Research Institute.

***Biochemistry Dept- Animal Health Research Institute.

This study was conducted to compare the effect following subacute and subchronic exposure to equitoxic doses (1/4 and 1/8 Of LD₅₀) of the two end-use products (EUP) of Profenofos (P) pesticide, from different manufactures, on some biochemical indices and histopathological feature of internal organs in male albino rats. One hundred male albino rats were divided into six groups. First and fourth groups as received tap water as a control groups. Second and third groups were orally administered with Selecrone (Ps) and Cord (Pc) at the dose of 46.3 and 44.62 mg/kg body weight per day, respectively, (4 doses/week) for 28 days (subacute treatment). Fifth and sixth groups were dosed with (Ps) and (Pc) at the doses of 23.14 and 22.31 mg/kg body weight per day, respectively, (4 doses/week) for 60 days (Subchronic treatment). Liver and kidney functions and histopathological feature of internal organs (liver, kidneys, lung and brain) were examined. an administration of Animals with (Ps) and (Pc) at the dose of 1/4 LD₅₀ for 28 days revealed significant decrease in plasma AchE activity, while an elevation in the activity of AST and ALT enzymes were occurred. In addition, Pc-treated group exhibited significant increase in total protein and albumin levels in comparison with the control group. While, Ps treated group showed significant increase in albumin and urea levels. Significant decrease in AchE activity was observed in animals treated with (Ps) and (Pc) at the dose of 1/8 LD₅₀ for 60 days. On the contrary, (Ps) and (Pc) compounds did not alter AST and ALT activities, total protein, albumin, urea and creatine levels, except, AST activity and creatinine level increased in Pc-treated group. Degenerative changes, necrosis, inflammatory reactions and hemorrhage were the predominant histopathological alteration. In conclusion; both (Ps) and (Pc) caused biochemical and histopathological alterations of examined organs. The effect of (Ps) was more strength than (Pc) on liver, kidney, lung and brain tissues.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



14.45 - 15.00

Bilateral Ceruminous Gland Adenocarcinoma in a Cat

Bilateral Ceruminous Gland Adenocarcinoma in a Cat

Mahir AG Kubba¹, Saeed N. Wafa ²and Seham A. Al-Azreg ¹

¹ Department of Pathology, Faculty of Veterinary Medicine, University of Tripoli.

² Department of Surgery, Faculty of Veterinary Medicine, University of Tripoli.

Recurrent bilateral ceruminous gland adenocarcinoma was diagnosed in a nine years old Persian-mix female cat. The tumor nodules were first noticed in the external ear canal 5 years ago and were resected surgically 2 years after their discovery. Reoccurrence was further reported on two occasions, i.e at 7 months and 3 months respectively before the publication of this article and were surgically resected as well. The tumor cellular morphology was described and discussed. This article documents ceruminous gland adenocarcinoma in cat for the first time in Libya.

Saturday , 27/4/2013

1st Session

10.00- 10.30

Exfoliative Cytology: Principles and Practice

Exfoliative Cytology: Principles and Practice

Mary M. Christopher, DVM, PhD, Dipl ACVP, Dipl ECVCP

Professor of Clinical Pathology, University of California–Davis

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Guiding Principles

1. You don't know what you have until you look (or...you'll never meet a lesion not worth sampling)
2. Looking is learning
3. Garbage in, garbage out

Introduction

Exfoliative cytology (cytopathology) is a diagnostic tool in which individual cells are examined microscopically for the purpose of making a pathologic diagnosis. In veterinary medicine, cytologic specimens are usually prepared and stained using standard hematologic techniques. Cytology is often used as a screening test, to help guide or direct additional testing. However, in many cases, a definitive or etiologic diagnosis can be obtained. Cytology also is used to stage neoplasia (eg, lymphoma) and to evaluate metastases. In the US, veterinarians often examine cytology specimens in their practice but also send specimens to diagnostic laboratories for examination by a board-certified clinical pathologist. Cytology is used most often in small animal practice, but the principles and diagnostic applications are similar in all species.

Advantages and Limitations

Exfoliative cytology has important advantages over other diagnostic techniques, such as biopsy. Samples usually are easy to obtain, and the procedure is inexpensive, can be done relatively quickly, does not require special equipment or stains, is less invasive than biopsy or surgery, and is reasonably accurate. For some types of lesions, such as inflammation and round cell tumors, cytology yields superior diagnostic results compared with histopathology. Cytology also has important limitations. Because individual cells (and not tissue) are examined, tissue structure is not usually apparent; for some types of lesions, tissue structure may be essential to making a definitive diagnosis. Cytologic specimens also may not be representative because of overlying inflammation, heterogeneity of lesions, focal lesions, or low cellularity (low cell yield).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Methods

Exfoliative cytology can be applied to most tissues and fluids. Samples can be obtained using fine needle aspiration, impression smears, scrapings, or swabs. Ultrasound can be used to guide needle placement in internal organs. Smears are prepared using push-slide or squash-prep techniques, air-dried, and stained with a hematology (Romanowsky) stain. Rapid hematologic stains, such as Diff-Quik, are practical in the practice setting. New methylene blue is a good complementary stain for evaluating lipid, fungi, and alcohol-soluble structures. A good quality specimen should be cellular (although low-cellularity specimens are sometimes diagnostic). Cells should be spread in a monolayer (but not disrupted), and free of artifact (such as ultrasound gel) and excess blood. Sample quality is directly related to the quality of diagnostic results.

Microscopic examination of a cytologic specimen should include evaluation of overall cellularity, background material, the distribution and features of the predominant cell type, other cell types, non-cellular features (such as extracellular matrix, crystals, etc.), and organisms. Lesions are classified as degenerative, inflammatory, or proliferative, or a combination of these. Degenerative lesions include cysts, hematomas, and seromas; they often have a proteinaceous background, low cellularity, and a few macrophages.

Cytologic Evaluation of Inflammatory Lesions

Inflammatory lesions can be septic (infectious) or nonseptic. Septic inflammation can be caused by bacteria, fungi, or protozoa, all of which (with the exception of mycobacteria) are visible with Romanowsky stains. The type of inflammation (suppurative/ purulent, pyogranulomatous, granulomatous, eosinophilic, lymphoplasmacytic) can provide a clue as to the underlying etiology. Degenerate (karyolytic) neutrophils are often seen in septic inflammation. Follow-up culture or serology are often indicated.

Cytologic Evaluation of Proliferative Lesions

A proliferative lesion usually consists of a single cell type that can be classified as epithelial-glandular, mesenchymal, hemic, or neural tissue. Epithelial-glandular lesions (eg, skin, adnexa,

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



lung, liver, kidney, mammary gland) usually exfoliate well and yield high-cellularity samples. Epithelial cells form cohesive clumps and clusters with distinct cell borders. Mesenchymal (connective tissue) lesions do not usually exfoliate well, resulting in low-cellularity samples. Most types of mesenchymal cells (eg, fibroblasts, osteoblasts, synovial cells) produce extracellular matrix. Mesenchymal cells are found individually or in loose aggregates and are often spindloid and have indistinct cell borders. Hemic tissue (eg, bone marrow, spleen, lymph nodes) exfoliates well so samples are usually highly cellular. Hemic cells are round and discrete and have high N:C ratios. Neural tissue is found in brain or spinal cord lesions.

Proliferative lesions are classified as hyperplastic (normal tissue), metaplastic (tissue that has changed from one cell type to another), dysplastic (abnormal tissue), or neoplastic. Neoplasia is further categorized as benign or malignant based on cellular criteria of malignancy, including anisocytosis, hyperchromicity, high N:C ratios, anisokaryosis, mitotic figures, nuclear molding, and multiple, prominent, pleomorphic nuclei and nucleoli. Hyperplasia and benign neoplasia appear similar cytologically. Secondary inflammation can cause cellular dysplasia, confounding a diagnosis of neoplasia. Malignant neoplasms include carcinomas (epithelial-glandular origin), sarcomas (mesenchymal), round cell tumors (hemic), and melanomas (neuroectodermal). Immunocytochemical stains are used to further characterize the cell of origin.

Summary: Exfoliative cytology is a useful diagnostic and learning tool. It is accurate, but also has limitations. Differentiating among degenerative, inflammatory, and neoplastic lesions can help guide additional testing and treatment.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



2nd Session

10.45- 11.15

New trends in teaching practical veterinary histology and pathology

New trends in teaching practical veterinary histology and pathology

Abdelrazik M.

Department of Cytology & Histology, Faculty of Veterinary medicine, Cairo University, Egypt.

In popular science imagery (newspapers, magazines, television drama, movies), the microscope remains the main symbol of the scientist and this is probably well justified. The microscope is the most widely used scientific instrument and it is hard to imagine a science laboratory without microscopes. Virtual histology and pathology teaching programs can replace “hands-on” microscopy with many benefits, including distance learning, and can result in considerable savings in teaching budgets making it a new and attractive approach for both veterinary medical staff and students. So what are the basic components of practical virtual microscopy lab? What are the advantages and disadvantages of virtual microscopy? What are the practically facing barriers that limit application of virtual histology and virtual pathology to teach students?

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Poster Presentations

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Poster Presentations

Poster No. 1

Encephalomyelitis in Sheep due to Sarcocystis sp

Yesari Eroksuz¹, Cem Ecmele Saki², Murat Dabak³, Ersoy Baydar³, Hatice Eroksuz¹ Aydin Çevik¹

¹Department of Pathology, Faculty of Veterinary Medicine, Firat University, 23200 Elazig, TURKEY

²Department of Parasitology, Faculty of Veterinary Medicine, Firat University, 23200 Elazig, TURKEY

³Department of Internal Medicine, Faculty of Veterinary Medicine, Firat University, 23200 Elazig, TURKEY

The aim of this study was to report a naturally occurring encephalomyelitis due to *Sarcocystis* sp. in a flock of 250 sheep. Eighty-two of the animals showed the neurological findings including hindlimb weakness, unable to rise without help, dog sitting and depression in 8 weeks period. They all either died or killed. Six animals exhibiting clinical signs were examined macroscopically and microscopically. No remarkable macroscopical changes were detected. Generally older sheep were affected being 79 of the animals were more than 4-years-old. Both the rams (7/7) and pregnant sheep were affected. There was no any remarkable changes in complete blood count. Serum biochemical analysis (n:13) indicated the elevated creatine kinase activity (188.23 IU/L, ref. value:7.7-101.0 IU/L), otherwise other enzyme values were in normal ranges. Histopathologic examinations revealed mild menigitis, mild focal perivascular cuffing and *Sarcocystis* sp. schizonts in brain and medulla spinalis in all cases. There was cystic and schizont form of *Sarcocystis* sp in the tongue, esophagus and heart sections. The cysts of *Sarcocystis ovicanis* were identified from muscle samples from tongue and heart in parasitologic examinations.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Poster No. 2

Experimental induction of hyperthyroidism in female Baladi goats with special reference to some biochemical parameters

A.M. Bakeer*, Iman B. Shaheed*, Sherein, S.A El Gayed*, Reda M.S. Korany* and Naela M. Ragaa**

*Pathology Department, Faculty of Veterinary Medicine, Cairo University, Egypt.

**Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Cairo University.

This study was conducted to investigate the effect of experimental induction of hyperthyroidism on different body organs of Ten female Baladi goats, five months old as an animal model through determination of hormonal assay especially T3 and T4 and monitoring the pathological changes. Levothyroxine sodium at the rate 1300 µg/animal/day as oral drench was used to induce hyperthyroidism for four weeks. Hormonal assay of this group showed significant increase in both T3 and T4 levels, whereas total lipid showed significant decrease in its level. The inducted animals showed round areas of alopecia at the face and ear with emaciation. Gross examination of the sacrificed goats which were treated with levothyroxine sodium showed large pale kidneys and distention in the gall bladder. The histopathological investigations of kidneys revealed vacuolar degeneration in the epithelium of renal tubule and endothelium of glomerular tuft. Liver of goats with induced hyperthyroidism showed vacuolar degeneration and necrosis of hepatocytes. Microscopic examination of the thyroid glands of affected cases revealed large dilated follicles with abundant colloid, the lining epithelium of the follicles were low cuboidal to squamous (colloid goiter).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



From these results we concluded that, Experimental hyperthyroidism has moderate effects on different body organs (kidneys, liver, thyroid glands and skin) and also some blood parameters (T3,T4 and total lipid). Also hyperthyroidism induced colloid goiter.

Poster No. 3

Preliminary Pathological Study on Equine Stomach

Asmaa Khiry , Rawhia Esawy Doghaim and Kawkab Abdel Aziz Ahmed

Department of Pathology, Faculty of Veterinary Medicine, Cairo University

This study aimed to highlight the naturally occurring lesions in equine stomach. In the current study 44 animals (35 donkeys and 9 horses) were used, after euthanasia the stomach was removed from the attached viscera, opened along its greater curvature and the contents were washed out by running cold water then prepared for histopathological examination.

The results of gross examination revealed hyperemia, edema, erosion, ulcers as well as gross parasitic lesion.

The results of histopathological examination were as follows:

A) Within the non- glandular region the lesion included:

Hyperkeratosis, acanthosis, vacuolar degeneration, gastritis represented by hyperemia, edema, erosions, ulceration, scaring and angiogenesis in addition to gasterophilosis.

B) At the margo plicatus the lesions included:

Hyperkeratosis, vacuolar degeneration, inflammation characterized by hyperactivity of mucus glands up to periglandular fibroplasia as well as glandular metaplasia.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



C) Within the glandular region:

Gastritis was shown by hyperemia, hemorrhage, glandular dysplasia and periglandular fibroplasia as well as parasitic infestation (*Habronema spp.* and *Draschia megastoma*)

This study is a preliminary one as an overview of gross and microscopic characters irrespective to etiology, age, sex or seasonal variations. More advanced study is recommended for correlation between lesions and their related factors especially causative agent.

It was obvious that the lesions did not differ either in horses or donkeys and there were no species specific lesions detected. The main difference was in the severity of lesions which was more severe in donkeys.

Poster No. 4

MSCs treat liver fibrosis and cirrhosis

Fatma Ahmed, Nabiha Ramadan, Iman, B. Shaheed

Department of pathology, Faculty of veterinary Medicine, Cairo University, Cairo 12211, Egypt

Objective:-

Cirrhosis is a common irreversible problem, in the late stages the only treatment is liver transplantation. In this regard, the administration of Mesenchymal stem cells (MSCs) was used to treat liver cirrhosis through reducing inflammation, collagen deposition, and remodeling. Furthermore, MSCs was used to protect liver against progression of fibrosis to cirrhosis.

Methods:-

Sixty male albino rats (average weight of each rat ca. 200-250 g) divided into two groups: First group (40 rats) was used for induction of cirrhosis by CCl₄ administration and the second group was control negative group. After 42 days, three animals from first group infused with

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



MSCs (isolated from bone marrow of 4 weeks male albino rats, with concentration ca. 3×10^6 cells), while CCl₄ administration stopped to estimate treatment effect of MSCs (i.e., group A). Moreover, at day 56, another three rats isolated from first group and then infused with MSCs, beside CCl₄ administration to estimate the treatment effect of MSCs in presence of CCl₄ (i.e., group B). In addition to, three rats from second group (control negative) injected with MSCs and used for induction of cirrhosis to estimate protective effect of MSCs (i.e., group C).

Furthermore, at day 63, three other animals from first group isolated and injected with MSCs and CCl₄ administration stopped to determine the treatment effect of MSCs (i.e., group D). On the other hand, All animals are sacrificed at day 93, and liver tissue is collected for histopathology and immunohistochemistry.

Results:-

MSCs treatment show significant improvement in liver of treated groups, amount of collagen decreased, degree of inflammation decreased, and remolding occurred. For instance, fibrosis was detected after 42 days of CCl₄ administration. After, injection of these rats with MSCs for 4-6 weeks resulted in:

- i. About 100% improvement of group (A) which was treated with MSCs, 70% improvement of group (C) and about 60 % improvement of group (D) was treated from fibrosis.
- ii. While, 60 % of group B was protected from fibrosis in comparison with control groups.

Conclusion:-

In comparison with control negative, the improvement in treated groups is observed, amount of collagen decreased, degree of inflammation decreased, and remolding occurred. In

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



other words, Administration of MSCs used to treat liver fibrosis and protect against its progression.

Poster No. 5

Histopathological evaluation to anticarcinogenic efficacy of some antioxidant agents in Mice

Iman, B. Shaheed

Department of pathology, Faculty of Veterinary Medicine, Cairo University. Egypt

This study was conducted to evaluate the anticarcinogenic efficacy of some of antioxidant through histopathological examination of internal organs of mice treated with ethylene thiourea (ETU) which is potent carcinogenic compounds. One hundred male mice were assigned to ten groups 10 mice each. The 1st group served as negative control whereas the 2nd group receives 300mg/ 1 ETU daily in drinking water for 24 successive weeks. Groups (3) to (6) received ETU and treated with antioxidant agent for 24 successive weeks. Group (3) treated with oily garlic preparation while group (4) received lyophilized yoghurt Whey. Group (5) treated with combination of Selenium and Vitamin E while group (6) supplied with Vitamin A in combination with Vitamin C. Groups (7-10) received in their diets only the antioxidant agents daily for 24 successive weeks. For histopathological examination five mice from each group were sacrificed at 12 and 24 weeks of experiment and tissue specimens were obtained from liver, kidneys, urinary bladder, stomach and thyroid gland. The histopathological examination of group (2) which treated with ETU revealed high incidence of tumor formation in different organs including cholangiocarcinoma and cholangiocarcinoma in liver, tubular adenocarcinoma in kidneys, transitional cell carcinomas in renal pelvis and bladder , papilloma in bladder and squamous cells carcinoma in non-glandular part of stomach while adenocarcinoma was observed

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



in glandular portions. Thyroid gland showed hyperplasia of follicular cells and adenocarcinoma. Groups received ETU and antioxidants (3-6) showed low incidence of tumor formation in some organs with mild to moderate degenerative changes in different internal organs and hyperplastic activity in renal pelvis, urinary bladder, stomach and thyroid gland. It was concluded that antioxidants has a great role in minimizing the carcinogenic and the toxic effect of ETU in internal organs of mice especially treated with oily garlic and yoghurt whey.

Poster No. 6

Anti -DNA antibodies specific to Cucumber mosaic virus coat protein induce autoimmune Lupus nephritis in Mice

Iman B. Shaheed^{1*}, Haggag S.Zein², Jaime A.Teixeira da Silva³ and Kazutaka Miyatake⁴

¹Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt.

²Department of Genetics, Faculty of Agriculture, Cairo University, Egypt.

³Faculty of Agriculture and Graduate School of Agriculture, Japan.

⁴Department of Applied Biological Chemistry, Graduate School of Agriculture and biological Sciences, Japan.

Auto antibodies against double stranded (ds) DNA are not only a helpful serological marker for diagnosis of systemic Lupus Erythematosus but have also been shown to be crucial in the pathogenesis of Lupus nephritis. Plant virus particles have been used to express a number of animal B- cells epitopes, which can be used in vaccine to confer protective immunity against human and animals diseases. This study was conducted to examine the pathological changes induced in kidneys of mice after immunization by Cucumber mosaic virus coat protein (CMV-

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



CP) subgroup I. A total of 10 hybridomas were generated from five fusions of BALB/ C mice. For histopathological examination, kidneys of mice were collected, fixed in 10% formol saline and processed by convention methods; also immunohistochemistry stain was used for detecting the immune deposit. Monoclonal antibodies (mAb) specific to CMV- CP were designed from cDNA and deduced amino acids sequences of the light chains of hybridomas cell lines. mAb showed DNA- hydrolytic activity at an optimum pH of 4-5, which is a typical pattern of autoimmune diseases in which the autoantibodies hydrolyze supercoiled plasmid DNA. The histopathological examination of mice kidneys revealed signs of Lupus nephritis in which there were proliferative and membranous glomerulonephritis. Degeneration and necrosis of renal tubules were observed, it replaced by fibrin exudates and inflammatory cells. Lupus Erythematosus cells were recorded in tubulointerstitial tissues. The autoimmune deposit was detected by immunohistochemistry in mesangial cells, wall of blood vessels, inflammatory cells and tubulointerstitial tissues. This is the first evidence ever that CMV-CP could stimulate catalytic antibodies, which have identical sequence homology with autoantibodies and induce Lupus nephritis in mice kidneys. Furthermore, The CMV-CP specific mAbs will be important for isolating antibodies specific to CPs of bacteria viruses and cancer cells, etc. that could be used for medical therapy.

Poster No. 7

Self-Assembling of Gold Nanoparticles Array for Electro-Sensing and drug Delivery Applications

Gumaa A. El-Nagar¹, Ahmad M. Mohammad^{1,2}, Mohamed S. El-Deab^{1,3},
Bahgat E. El-Anadouli^{1,*}

¹Chemistry Department, Faculty of Science, Cairo University, Cairo 12613, Egypt

^{2,3} Department of Chemical Engineering, Faculty of Engineering, The British University in Egypt, Cairo 11837, Egypt

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Materials in nanosize exhibit unique physical and chemical properties because of their size, morphology and large surface area in contrast to bulk materials. They have great potential applications in many fields such as catalysis, chemical and biochemical sensing, and biological imaging. To date, gold nanoparticles (AuNPs) have received much attention when compared to other

metal nanoparticles mainly due to their ease of preparation in different shapes and sizes (rods, dots), high stability and their shape and size-dependent catalytic activity. Gold nanoparticles are defined as stable colloid solutions of clusters of gold atoms with sizes in the nanometer scale. At this nanoscale, AuNPs possess different physicochemical characteristics when compared to the bulk gold, most obvious example being the color change from yellow to ruby red when bulk gold is converted into nanoparticulate gold, adsorb and emitted light strongly so it can detected at low concentrations (diagnosis of cancer) and local heating (used in chemotherapy treatment). A colloidal solution of citrate-stabilized gold nanoparticles (AuNPs) with an average size of ca. 2.6 nm has been prepared, characterized and further implemented in electro-sensing and drug delivery applications. This colloidal solution of AuNPs has been prepared via the reduction of NaAuCl₄ with sodium tetrahydroborate (NaBH₄) using trisodium citrate as a stabilizer. The optical properties of this solution have been studied with UV–Vis spectroscopy. Next, these AuNPs have been immobilized onto a polycrystalline Au (poly-Au) electrode with the assistance of benzenedimethanethiol (BDMT), which

served as a binder. Attention has been taken to ensure the formation of a compact impermeable layer of BDMT on poly-Au electrode, in order isolate the ploy-Au surface from participating in the upcoming applications. Interestingly, the AuNPs-modified Au electrode has shown a better sensing capability for ascorbic acid than that of the bare poly-Au, which opens opportunities for future designing of nanoparticles-based biological sensors.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

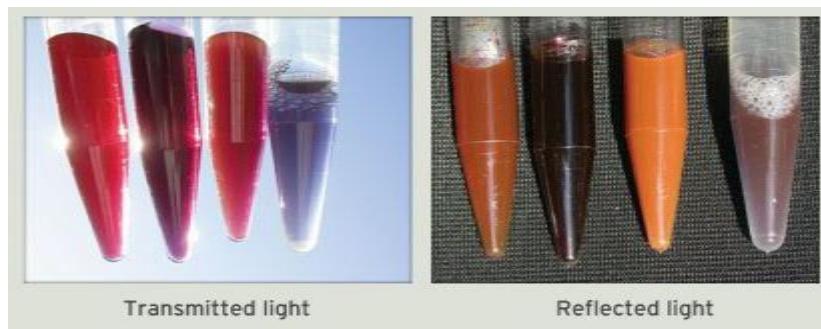
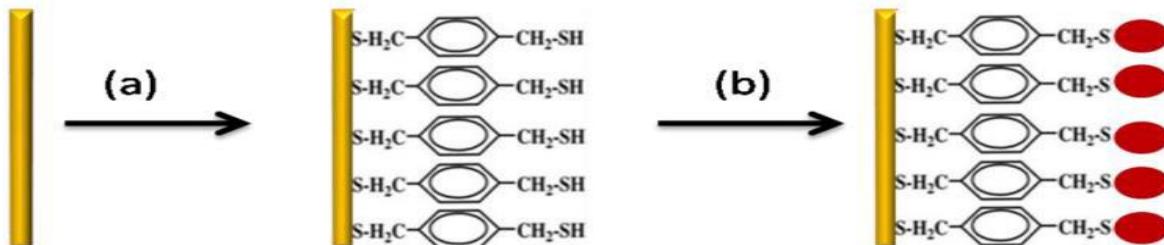
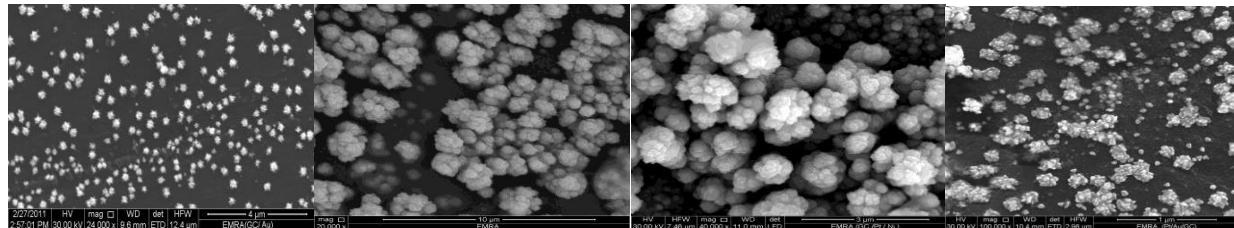


Figure : (A) FE-SEM images for AuNPs with different shapes, (B) Self-assembling of BDMT (a) and AuNPs (b), (C) UV-Vis spectra for the gold nanoparticles colloidal solution.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Poster No. 8

Clinical and pathological evaluation of dogs with three different superfacial neoplasms

Reda M.S. Korany* and Khaled M. Abd elhakim**

*Pathology Department, Faculty of Veterinary Medicine, Cairo University, Egypt.

**Police Officer, Police Academy, M.V.Sc., Faculty of Veterinary Medicine, Cairo University, Egypt.

In this study three types of dog neoplasms were reported at period from January, 2010 to March, 2013. These cases were admitted to K-9 department clinic at police academy and private clinic in Egypt. Clinical and histopathological examinations were performed for all cases. First case was male German Shepherd, five years old had an overgrowth about 3 cm in diameter at its checks, by clinical examination this mass was circumscribed, encapsulated, firm, lobulated, white and glistening. Histopathological examination revealed parotid adenoma formed of acini of variable sizes and shapes and lined by cuboidal epithelium with intact basement membrane. They formed papillary and cyst-papillary adenoma in some areas. Connective tissue stroma which contained well-developed blood vessels was found between the glands. Dog treated by surgical excision with no recurrence.

Second case was male German Shepherd, three years old had an overgrowth about 7 cm in diameter at its perineum, by clinical examination this overgrowth was soft, multiple, white or even congested lobulated masses of variable shapes and sizes. Microscopically, it was lipoma which characterized by large, fat cells with flattened and compressed nuclei (Signet ring). Each

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



group of cells was separated by scanty fibrous stroma with presence of prominent capsule; blood vessels in some areas were congested. Cure occurred by surgical excision.

Third case was female Great Dane, three years old had a perivaginal multiple, ulcerated and easily bled overgrowth which was highly infiltrating, Microscopically, the tumor was mastocytoma and composed of groups of mast cells (large cell with deeply eosinophilic granules and large eccentric basophilic nuclei) separated by hyalinized connective tissue stroma, by Toluidine blue stain granules appeared violet with blue background. Euthanasia performed due to high infiltration.

In conclusion, dog neoplasms are common especially in old dogs, and their types resemble human ones. This study explains three naturally occurring types of them.

Poster No. 9

Pathological and immunohistochemical studies in mice experimentally infected with trypanosoma evansi

Reham, Mahmoud, Ezz EL-Din Sakr and Magdi Mohamed el-Mahdi

Department of Pathology, Faculty of Veterinary Medicine -Cairo University , Gizza, Egypt.

This study was designed to provide comprehensive knowledge on histopathological alterations during the progress of the disease in liver, spleen, kidneys, lungs, heart, brain and testes of the mice infected with T. evansi, detecting the distribution of trypanosomal antigen in various tissues by using immunohistochemical technique, for this purpose eighty mice were infected with 104 Trypanosoma evansi by I/P injection. Tissue samples (liver, spleen, kidneys, lungs, heart, brain and testes) were collected from mice scarified at 3, 5, 7, 10, 12, 15, 17, 22 day

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



post inoculation for histopathological and immunohistochemical examination. Gross post-mortem examination revealed enlargement of spleen, congestion of liver. Tissue sections revealed presence of numerous trypanosomes in blood vessels of liver, spleen, kidneys, lung, heart, brain and testes. Microscopically, liver revealed lesions varying from vacuolar degeneration, coagulative necrosis along with congestion and haemorrhages. Spleen showed extensive hemorrhages in red pulp area, extramedullary hematopoiesis and lymphoidal necrosis. Lungs revealed oedema, congestion and mild inflammatory changes. Heart revealed mild degenerative changes along with interstitial oedema. Brain revealed mild degenerative changes along with congestion of meningeal blood vessels. Testes revealed severe testicular degeneration. All changes were consistent with trypanosome infection and were confirmed by presence of trypanosomes in most of the tissue by immunohistochemical technique.

Poster No. 10

Toxico-pathological study on nitrate in goats

Iman, B. Shaheed¹ and Manal, M. Makhlof²

¹Department of Pathology, Faculty of Veterinary Medicine -Cairo University , Gizza, Egypt.

² Department of Biochemistry Animal Health Research Institute, Dokki, Giza

This study was conducted to study the adverse effect of nitrate on some endocrine glands of goats (thyroid, parathyroid and adrenal) through monitoring histopathological changes and abnormalities in hormonal assay and haemogram profile. Eight goats were divided equally into two groups; the first one served as control whereas the second group received 5 mg/ kg B.W. potassium nitrate per day orally for 30 successive days. At the end of experiment, blood samples were taken to determine the values of Triiodothyronine (T3), Thyroxin (T4), cortisol, and calcium

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



as well as haemogram profile. Tissue specimens were collected from thyroid, parathyroid and adrenal glands in addition to mesenteric and thoracic lymph nodes for histopathological examination. There were significant decrease in T3 and T4 levels; meanwhile there were increase in cortisol and calcium levels in serum of treated group in comparison to values of control one. A significant decrease in hemoglobin concentration ,Packed cell volume , Mean corpuscular volume , Mean corpuscular hemoglobin , Red blood cells count , and Mean corpuscular hemoglobin concentration were also detected. Total leukocytic count showed significant increase with moncytosis, neutrophilia and lymphopenia. The histopathological examination of thyroid gland in treated goats revealed signs of goiter represented by hypertrophy and hyperplasia of follicular cells. The follicles appeared irregular in size and shape and containing varying amount of colloid. The chief cells of parathyroid gland showed focal hypertrophy and hyperplasia with increased eosinophilic or vacuolated cytoplasm. Adrenal cortex showed hyperplasia and hypertrophy of zona glomerulosa , zona fasciculate and zona reticularis. Hyperplastic nodules of chromaffin cells were detected in adrenal medulla. There was depletion in lymphoid follicles in the cortex of lymph nodes. From the current study we concluded that nitrate induces morpho-functional alterations in thyroid, parathyroid and adrenal glands as well as hypochromic and microcytic anemia with immunosuppressant action.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Manuscripts

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



ABSCESSSES IN DROMEDARY CAMELS, SHEEP AND GOATS ETIOLOGY AND PATHOLOGY

Zidan, K.H¹; Mazloum, K.²; Saran, M.A.², and Hatem, M.E.³

¹ Al-Riyadh Diagnostic Veterinary Laboratory, ² National Agriculture& Animal Resources Research Center, Ministry of Agriculture, Kingdom of Saudi Arabia 11195 and ³ Fac. of Vet. Medicine, Cairo Univ., Egypt.

ABSTRACT

Abscesses in farm animals cost the producers tremendous losses. Forty five samples were collected from dromedary camels in addition to two hundred and fifty samples collected from sheep and goats from Al-Riyadh, Kingdom of Saudi Arabia during 2012. Bacteriological examination of the samples revealed that the main causes of camel abscesses were *Corynebacterium* species (39%) and *Staphylococcus aureus aureus* (17.1%). Streptococci and *Rhodococcus* were also isolated from few cases, while no aerobic and/or anaerobic bacterial growth was seen in 29.3% of the collected samples. Four subcutaneous dermoid cysts of camel samples were collected and described during the investigation. These cysts were filled with hair and brownish watery fluid. On the other hand the main causes of abscessation in sheep and goats were *Staphylococcus aureus anaerobius* (55%), *Corynebacterium pseudotuberculosis* (24.5%) and *Staphylococcus aureus aureus* (15%). The histopathological picture of the affected camel lymph nodes (LN) infected with *Corynebacterium* was quite different from that appeared in caseous lymphadenitis (CLA) of sheep and goats.

The current study showed that the infection of dromedary camels with abscesses is comparatively little and self limiting; unlike that in sheep and goats.

Key words: dromedary camel, sheep, goat, abscess, dermoid cyst, histopatholgy.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



INTRODUCTION

Abscesses infection of farm animals is detrimental to the livestock due to the tremendous economic losses of animals, meat, skin, and wool production associated with this affection (**Paton et al., 1988 and Alharbi and Mahmoud, 2012**). Sheep and goat industries worldwide suffer significant economic losses because infected animal have to be culled from breeding flocks due to poor physical condition or decreased fertility and condemnation of carcasses totally or partially at abattoirs (**Connor et al., 2000 and Hassan et al., 2011**).

Camel infections with pyogenic bacteria such as *Corynebacterium pseudotuberculosis*, *C. pyogenes*, group B streptococci and staphylococci have been reported and common in many areas. Abscesses are common in dromedaries, particularly in the form of lymphangitis accompanied by suppurative lymphadenitis of cervical and sciatic lymph nodes (**Fassi-Fehri, 1987**). Camel abscess in the pre-pectoral lymphglands, at the base of the neck, is a common finding in almost every camel. Abscesses of the other camel superficial LNs like prescapular and precrural are also common (**Abdurahman and Bornstein, 1991**). The causative organism enters through the damaged skin and mucous membrane and finally reaches the regional lymph node and causes inflammatory and necrotic changes (**Simmon et al., 1998**).

Corynebacterium pseudotuberculosis is an important animal pathogen. It is the etiological agent of a disease that is commonly called caseous lymphadenitis (CLA) or cheesy gland in sheep (**Williamson, 2001**). This disease is found in the entire world's major sheep and goat production areas, causing significant economic losses (**Paton et al., 2003; Williamson, 2001**). Staphylococci are widespread in nature and occur as a normal inhabitant of skin (**Mustafa, 1987**). They may infect wounds and be present in cutaneous abscesses and lesions. Morel's disease is caused by *Staphylococcus aureus* subsp. *anaerobius* (**Szaluś-Jordanow et al., 2010**). The bacterium was first isolated in 1920 (**Aynaud, 1922**). *Staphylococcus aureus* subspecies *anaerobius* is a microaerophilic, catalase-negative bacterium (**de la Fuente et al., 2010**). The first report of Morel's disease in Saudi Arabia in goat was by **Alhendi et al. (1993)**. Morel's

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



disease leads to formation of abscesses located mainly in close association with superficial lymph nodes mostly in the head region (**Szalus-Jordanow et al., 2010**).

The current study was done in order to determine the main bacterial causes of abscesses in farm animals (camels, sheep and goats) in Al- Riyadh region, Kingdom of Saudi Arabia.

MATERIALS AND METHODS

Forty five samples were collected from camel's skin infections and the draining LNs after slaughtering. Four samples were described as dermoid cysts, so the abscess samples were 41. Camel samples were found only in the superficial LNs or skin of the head and neck. Two hundred and fifty samples were collected from sheep and goats. Fifty pus samples and swabs were collected from the affected live animals from different farms suffering from the abscesses and 200 samples were collected from the abattoir in Al-Riyadh. Sheep and goat abscesses were found in many parts of the carcass (superficial LNs, visceral LNs, muscles, liver, and lungs). All samples were cultured onto sheep blood agar and incubated aerobically and anaerobically for 48 hours at 37°C.

The identification of *S. aureus* subsp. *anaerobius* was initially performed based on the growth in anaerobic conditions and lack of growth in aerobic conditions, microscopic examination (presence of Gram-positive cocci) and biochemical features (positive coagulase test, negative catalase and absence of clumping factor). Distinguished from *Staphylococcus aureus* subsp. *aureus* by lack of pigment and clumping factor (**Schleifer and Bell 2009**). The identification of each isolate was confirmed biochemically by using Biolog™ system (Biolog, Hayward, USA) AN microplate for anaerobic bacteria (Cat. No. 1007). *C. pseudotuberculosis* was identified based on microscopic examination (presence of Gram-positive, pleomorphic rods, singly or in pairs, often in "V" formation, creating "Chinese letters" and biochemical properties testing using the analytical profile index (API) Coryne System (API-bioMérieux, Inc., France) including strong positive catalase test. The other bacteria were identified morphologically (Gram staining

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



and examined microscopically), and identified biochemically using API system BioMérieux and/or Biolog™ system using GEN III microplate for aerobic bacteria (Cat. No. 1030). Sections from the infected camel lymph nodes were histopathologically examined for changes.

RESULTS

The causes of camel abscesses were *Corynebacterium* species 16/41 (39.0%), *Staphylococcus aureus* 7/41 (17.1%), *Streptococcus* species 3/41 (7.3%), *Rhodococcus equi* 2/41 (4.9%); moreover 12/41 (29.3%) of the collected samples didn't show any bacterial growth up to 96 hours after incubation (Figure 1). The *Corynebacterium* isolates were identified as 3 isolates were *C. ulcerans*, 2 isolates were *C. pseudotuberculosis*, 2 isolates were *C. xerosis*, one isolate was *C. testudinoris* and the other 8 isolates were recorded as unidentified *Corynebacterium* species. Also, four subcutaneous dermoid cysts (were collected unintentionally from camels as suspected abscesses) filled with hair, cornified cells and sanguineous watery fluids representing 4/45 (8.9%) of the collected samples (Photo. 1). Sheep and goat abscesses caused by *Staphylococcus aureus anaerobius* (55%), *Corynebacterium pseudotuberculosis* (24.5%), *Staphylococcus aureus aureus* (15%) and other bacteria including staphylococci, streptococci, and *Pasteurella* species representing 5.5% (Figure 2).

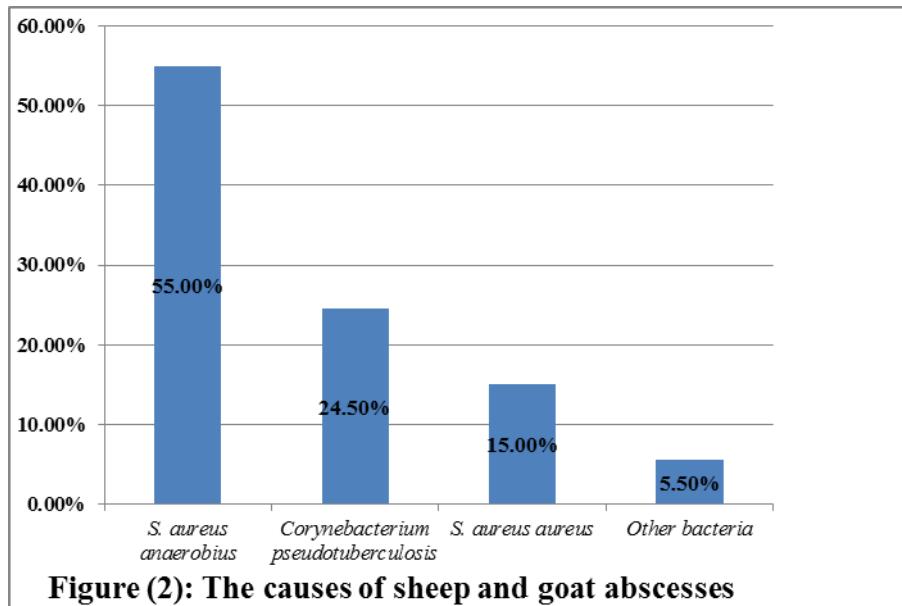
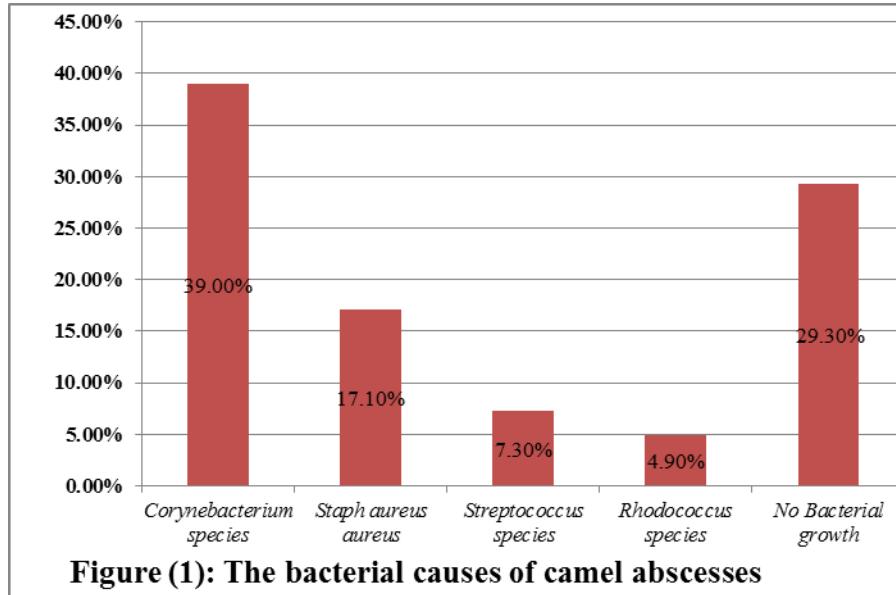
The camel's lymph nodes were enlarged and few lobes on cut section appeared red and hemorrhagic. Some cases showed pus in the LN center (Photo. 2). The histopathological examination of the affected lymph nodes revealed signs of hemorrhagic and suppurative lymphadenitis (Photo. 3). Liquifactive necrosis and pus were seen in the cortex and medulla. The pus appeared as homogeneous eosinophilic fluids with large numbers of neutrophils in addition to many macrophages and plasma cells. Also large areas of hemorrhages were recorded among the tissues of the lymph nodes. Hemosiderin pigment was noticed distributed among the tissues of the lymph nodes. Also depletion of lymphoid follicles in the cortex was prominent. The blood vessels appeared thickened and infiltrated by inflammatory cells.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Photo. (1): Shows closed dermoid cyst (1) and an open one (2).

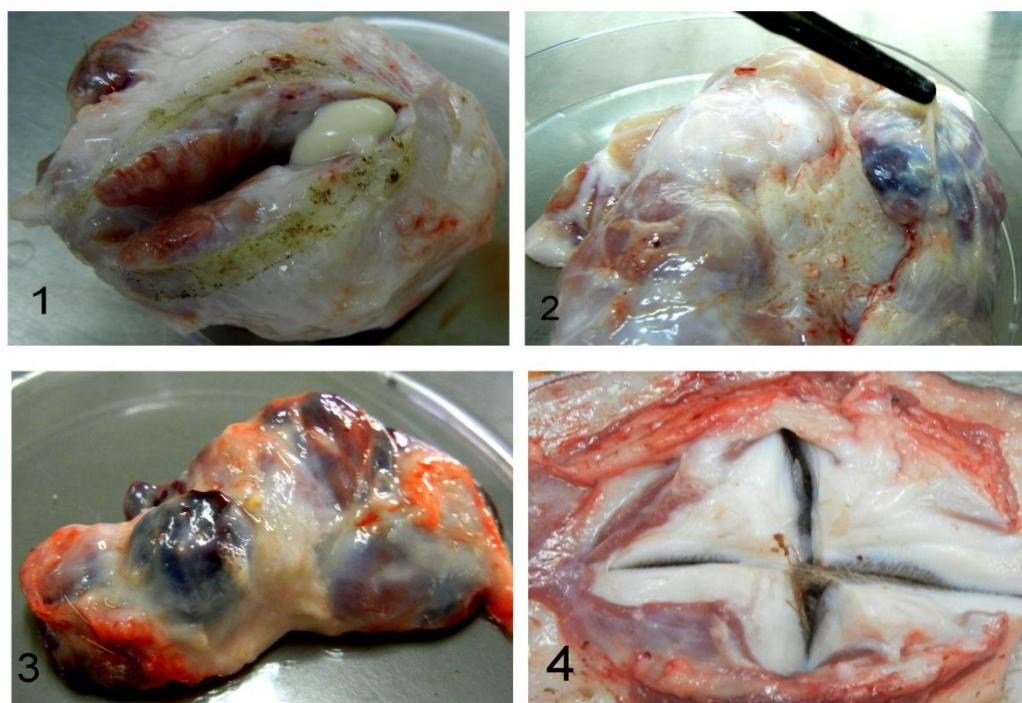
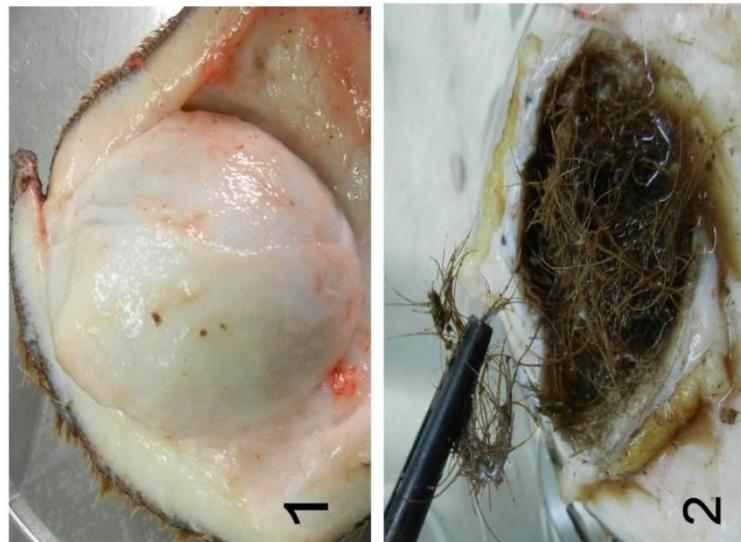


Photo. (2): Shows *Corynebacterium* species affected LNs 1, 2, and 3. Number 4 show site of skin affection as a hard thick mass of fibrous tissue.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

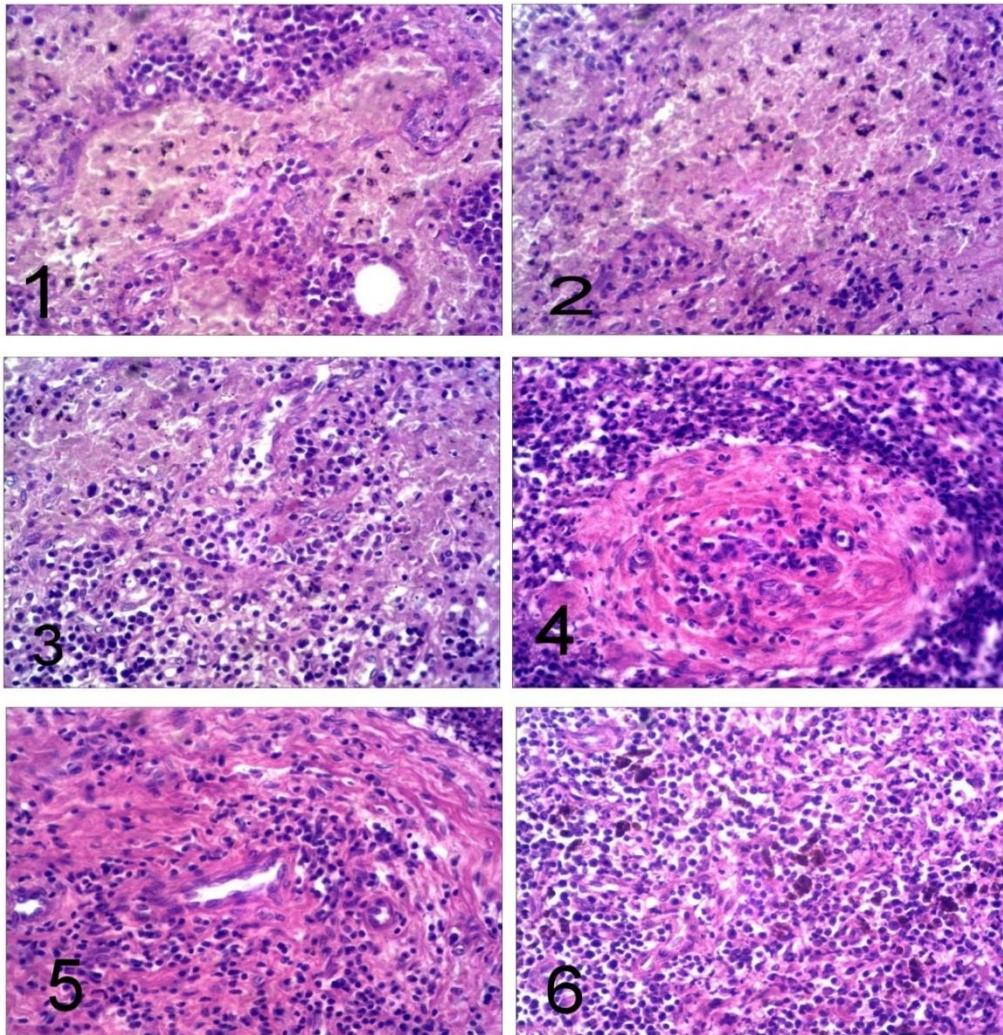


Photo. (3): Histopathological picture of camel LN: 1 and 2 show pus and inflammatory cells, 3 and 4 show inflammatory cells around the blood vessels and thickening walls, 5 and 6 show hemosiderin pigment in lymph nodes.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



DISCUSSION

Several livestock owners compliant to the authority of the Ministry of Agriculture, from the problem of abscesses in camel, sheep and goat, were the primary motivation for the present study. The aim of the current study was to determine the actual causes and the probable predisposing factors of this problem in the farm animals in the central region of the KSA.

Camel farm of about 80 animals in Al-Riyadh, KSA, most of them suffered from abscesses or localized enlargements in the head and neck regions of all animals. Trials were done to incise these lesions to take samples, but all lesions were fibrous tissue without any fluids oozing (Photo. 2). Samples from live animals were taken from the margin of each lesion using sterilized bacteriological swab. Veterinarians and meat inspectors in many abattoirs in Al-Riyadh area concluded that abscesses in camels and camel carcasses are comparatively rare than in cattle, sheep and goats. After repeated field and abattoirs visits, all of the recorded affected cases showed enlarged hard solid masses mostly at the base of the neck. The infection rate was 1- 2% mostly in the mature camels. In the live animals, some cases showed open lesions voiding bloody pus. The draining LNs, inferior cervical was the most affected, also the mandibular, the parotid, and the prescapular LNs were affected. The lesions of camel's superficial abscesses were detected and marked before slaughter. The marked samples were taken after slaughter of the animals. Incision of this enlarged masses showed a hard mass of fibrous tissue of the affected area of the skin as shown in photo. 2. The draining LN appeared enlarged and inflamed. Few lobes in the LN were hemorrhagic from the outside view and in cut sections. Abscesses mostly appeared in the skin near the LN and few cases showed abscess in the LN. The organism was isolated from the abscess and the draining LN. The *Coynebacterium* group in the present study was the primary cause of skin abscesses in the dromedary camels since it was recovered from 16/41 (39%) of the cases, however only few cases were identified as *C. pseudotuberculosis* 2/16 (12.5%). *C. ulcerans* was also recovered from 3/16 (18.75%) of the examined cases. This organism was previously reported as a cause of camel abscess by **Tejedor et al. (2000)**. Some

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



other *Corynebacterium* species were also recovered and identified. In the current study the disease picture in camels was different than that in sheep and goats in the fact that it was mostly self limiting and the organism was trapped at the site of infection and the adjacent drained LN regardless of the causative organism. Infection usually did not spread to other LNs. Moreover, *S. aureus* and *Streptococcus* species were recovered from 7 and 3 out of 41 cases, respectively.

In camel samples it was noticed that 29.3% of samples were bacteriologically negative when cultured onto blood agar both aerobically and anaerobically, a finding not seen in sheep and goat samples. This may be attributed to the chronic nature of camel abscesses in which the organisms may be dead.

The abscesses infection percentage in sheep and goats in most farms ranged from 2-7% and in few farms reached more than 20% depending on the management practices. *Staphylococcus aureus anaerobius* appeared as the primary bacterial cause of the sheep abscesses since it was isolated from 55% of samples. This finding is consistent with that of **Musa et al. (2012)**. *C. pseudotuberculosis* caused 24.5% of abscesses in sheep and goats examined during the present investigation. This percentage is more or less consistent with other reports in Saudi Arabia (**Alharbi, 2011**).

The use of the barbed wire fences for guarding the sheep, goats and camels may be one of the most important bad managerial practices that lead to wounds and sequentially introduction of infection (**Guimarães et al., 2011**). Camels may rub their heads and necks to the wire fences which predispose for abscess formation. Also the external parasites, mainly ticks, may also play a role in the etiology of abscesses (**Radwan et al., 1989**).

The macroscopic and microscopic picture of camel LNs affected with *Corynebacterium* species was completely different from that seen in sheep and goats. The lymph nodes of sheep and goat were characterized macroscopically by onion and lamellated appearance with central caseous

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



necrosis. Also microscopically the infection in sheep and goats was characterized by pyogranuloma in which there is centrally pus with inflammatory cells, surrounded by connective tissues. The lamellated appearance is due to presence of different layers of pus surrounded by connective tissue capsules (**Tehrani et al., 2012**).

The dermoid cysts in dromedary camels were reported in the current study since it represented 8.9% of the collected camel samples. These cysts were collected unintentionally as abscesses but by incision it was found that it is a subcutaneous sac filled with watery sanguineous fluids with cornified cells with a ball of hair (Photo. 1). Few reports of this skin anomaly in dromedary camels were previously recorded by **Purohit et al. (1989)** and **Oryan et al. (2010)**.

The abscess disease in sheep and goats can be controlled by avoiding the predisposing factors and probably by using a mixed autogenous vaccine from local isolates which is recommended for further investigations.

ACKNOWLEDGMENT:

The authors acknowledge Prof. Dr. Iman Shaheed for her help in the histopathological examination of the tissue samples

REFERENCES

- Abdurahman, O.Sh., and Bornstein, S. (1991):** Diseases of camels in Somalia and prospects for better health. Nomadic peoples (29): 104-112.
- Alharbi, K.B., and Mahmoud, O.M. (2012):** Abscess disease of sheep and goats: A disease of major concern in Saudi Arabia that urges production of an effective vaccine. J. of Agriculture and Vet. Sci., 5(2): 61-72.
- Alharbi, K.B. (2011):** Bacterial isolates from visceral abscesses of sheep at Qassim, Saudi Arabia. African J. of Microbiol. Res., 5(31):5622-5627.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Alhendi, A.B.; Al-Sanhousi, S.M.; al-Ghasnawi, Y.A.; Madawi, M. (1993): An outbreak of abscess disease in goats in Saudi Arabia. Zentralbl Veterinarmed A 40: 646-651.

Aynaud, M. (1922): La botryomycose du mouton. C R Acad Sci., 175: 1170-1172.

Connor, K.M.; Quire, M.M.; Baired, G., and Donachie, W. (2000): Characterization of United Kingdom isolates of *Corynebacterium pseudotuberculosis* using pulsed-field gel electrophoresis. J. of Clinical Microbiol., 38(7): 2633-2637.

de la Fuente, R.; Diez, R.M.; Dominguez-Bernal, G.; Orden, J. A., and Martinez-Pulgarin, S. (2010): Restoring catalase activity in *Staphylococcus aureus* subsp. *anaerobius* leads to loss of pathogenicity for lambs. Vet. Res., 41(4):41.

Fassi-Fehri, M.M. (1987): Diseases of camels. Rev. sci. tech. Off. int. Epiz., 6 (2): 337-354.

Guimarães A.S.; Carmo, F.B.; Heinemann, M.B.; Portela, R.W.D.; Meyer, R.; Lage, A.P.; Seyffert, N.; Miyoshi, A.; Azevedo, V., and Gouveia, A.M.G. (2011): High seroprevalence of caseous lymphadenitis identified in slaughterhouse samples as a consequence of deficiencies in sheep farm management in the state of Minas Gerais, Brazil. BMC Vet. Res., 7:68.

Hassan, N.A.; Al-Humiany, A.A.; Bahobail, A.S., and Mansoui, A.M.A. (2011): Bacteriological and pathological studies on caseous lymphadenitis in sheep in Saudi Arabia. International J. of Microbiol. Res., 2 (I): 28-37.

Musa, N.O.; Babiker, A.; Eltom, K.; Rodwan, K., and Sulieman M. El Sanousi, S.M. (2012): Prevalence of *Staphylococcus aureus* subsp. *anaerobius* in Sub-Clinical Abscess Cases of Sheep. British Microbiol. Res. J., 2(3): 131-136.

Mustafa, I.E. (1987): Bacterial diseases of dromedaries and bactrian camels. Rev. sci. tech. Off. int. Epiz., 6 (2): 391-405.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Oryan, A.; Hashemnia, M., and Mohammadalipour, A. (2010): Dermoid cyst in camel: a case report and brief literature review. Comparative Clinical Pathol., 21(5): 555-558.

Paton, M.W.; Mercy, A.R.; Wilkinson, F.C.; Gardner, J.J.; Sutherland, S.S., and Ellis, T.M. (1988): The effects of caseous lymphadenitis on wool production and body weight in young sheep. Australian J. of Vet. Res., 65: 117.

Paton, M.W.; Walker, S.B.; Rose, I.R.; Watt, G.F. (2003): Prevalence of caseous lymphadenitis and usage of caseous lymphadenitis vaccines in sheep flocks. Aust. Vet. J., 81:91-95.

Purohit, N.R.; Chouhan, D.S.; Dudi, P.R., and Vyas, U.K. (1989): Dermoid cysts in camels. Br Vet J., 145(1):89-90.

Radwan, A.; EL-Magawry, S.; Hawari, A.; AL-Bekaipdt, S.I., and Rebleza, R.O.M. (1989): *Corynebacterium pseudotuberculosis* infection in camels (*Camelus dromedarius*) in Saudi Arabia. Trop. Anita. Hlth Prod., 21: 229-230.

Schleifer, K.H. and Bell, J.A (). Family VIII. Family *Staphylococcaceae* In Bergey's Manual of Systematic Bacteriology, 2nd edition, volume III the firmicutes, Whitman, p: 401.

Simmon, C.P.; Dwtan, S.J.; Tachedlian, M.; Krywult, J.; Hodgson A.L.M., and Strugnell, R.A. (1998): Vaccine potential of attenuated mutant of *corynebacterium pseudotuberculosis* in sheep. Infect. Immun., 66(2): 474-479.

Szaluś-Jordanow, O.; Kaba, J.; Czopowicz, M.; Witkowski, L.; Nowicki, M.; Nowicka, D.; Stefańska, I.; Rzewuska, M.; Sobczak-Filipiak, M.; Binek, M., and Frymus, T. (2010): Epidemiological features of Morel's disease in goats. Polish J. of Vet. Sci., 13(3): 437-445.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Tehrani, A.; Javanbakht, J.; Hassan, M.A.M.; Zamani, M.; Rajabian, M.; Akbari, H. and

Shafei, R. (2012): Histopathological and bacteriological study on hepatic abscesses of Herrik sheep. J. Med Microb Diagn 1 Issue 4:115.

Tejedor, M.T.; Martin, J.L.; Lupiola, P., and Gutierrez, C. (2000): Caseous lymphadenitis caused by *Corynebacterium ulcerans* in the dromedary camel. Can. Vet. J., 41:126-127.

Williamson, L.H. (2001): Caseous lymphadenitis in small ruminants. Vet. Clin. North Am. Food Anim. Pract., 17: 359-371.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



EVALUATION OF SOME AVIAN INFLUENZA VACCINES ON COMMERCIAL LAYER CHICKS

M.M. Amer¹, Sherein, S.abdelgayed² and Abeer, A. Abd El-Baky ³

¹Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

²Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

³Department of Clinical Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

ABSTRACT

A total of 90 one day old commercial layer chicks were used for evaluating inactivated monovalent (H5N1) and bivalent (H5N1&ND) avian influenza vaccines. The chicks were divided equally into 3 groups as follow; A, B and C each group contains 30 chicks. The study continued for 5 weeks through which collection of samples was performed weekly. Feed intake (FI), body weight gain, feed conversion rate (FCR) and organ weight /body weight ratio were done. Immunological evaluation was carried out using haemagglutination inhibition antibody (HI) test against avian influenza virus (H5N1). Total erythrocyte and leukocyte counts, packed cell volume (PCV %), hemoglobin concentration and differential leukocytic count were performed to evaluate the clinico-hematological effect of AI vaccines. Serum total proteins, albumin, globulins, A/G ratio, uric acid and creatinine concentrations were done. In addition to hepatic enzymes activity and blood glucose level were carried to evaluate clinico-biochemical effect of AI vaccines. Examination of lymphoid organs (bursa, thymus and spleen) was done to evaluate histopathological effect of AI vaccines. Results of FI, body weight gain, FCR, organ weight /body weight ratio and HI antibody titers against avian influenza virus revealed, bivalent vaccinated group has higher values than monovalent vaccinated group. The clinicopathological changes revealed presence of significant leukocytosis due to significant lymphocytosis and significant hyperproteinemia due to hyperglobulinemia in both vaccinated groups but bivalent vaccinated group has higher values than monovalent vaccinated group. Both vaccines had no

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



effects on the other hematological parameters, blood glucose level, kidney and liver function tests. Histopathological findings of lymphoid organs confirmed that bivalent vaccine has immunological effects higher than that of monovalent vaccine. From the present study we concluded that, usage of inactivated bivalent (H5N1&ND) AI vaccine induced higher immunity than monovalent (H5N1) AI vaccine.

Key words: Clinical pathology, Immunology, Histopathology, Avian influenza vaccine.

INTRODUCTION

Avian influenza (AI) is one of the highly contagious Office of International Epizootics (OIE) list “A” diseases. The disease is also called “fowl plague” due to its high mortality in chicken. It has emerged as a disease with significant potential to affect commercial poultry production, resulting in extensive losses. On rare occasions, AI viruses have exhibited interspecies transmissibility to human (Sims et al., 2003). Human mortality has also been recorded due to H5N1 in Egypt. Avian influenza is caused by influenza “A” virus which belongs to family *Orthomyxoviridae* [Kilbourne, 1987]. It is a negative stranded, segmented RNA virus with 17 hemagglutinin and 10 neuraminidase types. H5N1 is the causative agent of avian flu and is endemic in many bird populations. The disease is characterized by nasal and lacrimal discharge, reddening of legs and comb, facial swelling, off feed and death. In Egypt during 2006, the outbreaks of high pathogenicity avian influenza (HPAI) virus of subtype H5N1 affected the major layer and broiler breeder as well as some broiler grandparent flocks was recorded. Different levels of control measures have been implemented to control the outbreaks that included condemnation of infected farms, strict bio-security and vaccination of commercial chickens [Swayne 2009]. Vaccination as a supportive tool in AI virus control strategies was implemented to limit spread of H5N1 and to reduce the losses [EFSA, 2008]. Different types of vaccines are already in use that decrease shedding of virus, morbidity, mortality and transmissibility through increasing resistance to infection and reducing field virus replication [Van den Berg, 2008]. Evaluation of different types of AI vaccines used in Egypt may provide

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



effective vaccination strategy. Moreover, the combinant vaccine of avian influenza and Newcastle disease (AI&ND) was recently recommended and commercialized for more protection against AI. The present study aimed to evaluate inactivated monovalent (H5N1) and bivalent (H5N1&ND) AI vaccines through its immune response, clinicopathological and histopathological effects on vaccinated layer chicks.

MATERIALS AND METHODS

Chicks and Experimental Design

A total of 90 one day old commercial layer chicks were used in this study. All of them were reared on floor housed system and were fed *ad libitum* on a balanced commercial ration. The chicks were divided equally into 3 groups as follow; A, B and C, 30 chicks each. Group A, non vaccinated control group. Group B, vaccinated with inactivated monovalent (H5N1) AI vaccine. Group C, vaccinated with inactivated bivalent (H5N1&ND) AI vaccine. The experiment continued for 5 weeks through which collection of samples was performed weekly.

Vaccination

Chicks of groups B and C were vaccinated with inactivated oil adjuvant monovalent (H5N1) AI vaccine obtained from Harbin Weike Biotechnology Development Company, China and bivalent (H5N1&ND) AI vaccine obtained from Veterinary Research and Vaccine, by subcutaneous route (S/C) at 9th day of age, respectively. Group A, considered as non vaccinated control group.

Haemagglutination inhibition (HI) test

The HI test was carried out in V-bottomed microtitre plate and 4 HA units of virus/antigen in 0.025 ml phosphate buffer saline [OIE-Manual, 2004], HI titres were given titer reference number according to Kaleta and Siegmann [Kaleta, and Siegmann, 1978].

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Chicken performance

Body weight (gm) and organ weight (gm) were recorded for each bird also, organ weight/ body weight ratio was calculated as described by Lucio and Hitchner [Lucio, and. Hithcner, 1980].

Blood Samples for Clinicopathological and Serological Examinations

Blood samples from 10 chicks of each group were collected at weekly intervals. Two blood samples were taken from each bird (wing vein). The first blood sample was anticoagulated by di-potassium salt of ethylene diamine tetra-acetic acid (EDTA) and used for evaluating hemogram. The second blood sample was collected in clean centrifuge tube and allowed to clot, then centrifuged at 3000 rpm for 10 minutes for serum separation. The clear non hemolysed supernatant serum was harvested for biochemical studies and haemagglutination inhibition (HI) test for determining serum antibody titers against AIV [Kaleta,, and Siegmann, 1978].

Hematological and Serum Biochemical Studies

Hematological Studies

Total erythrocyte and leukocyte counts were done using an improved Neubauer hemocytometer. Packed cell volume (PCV %) was estimated by microhematocrit technique. Hemoglobin concentration was colorimetrically determined using cyanmethemoglobin method. Differential leukocytic count was performed on Giemsa stained blood smears [Feldman,2000].

Serum Biochemical Studies

Serum samples were prepared to assay the following biochemical studies; serum total proteins was determined by the Biuret reaction according to Weichselbaum [Weichselbaum,, 1946], serum albumin was determined according to Dumas and Biggs [Dumas, and. Biggs,

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



1972] and serum globulins were determined by subtracting value of serum albumin from the value of serum total proteins. A/G ratio was obtained by subdividing values of serum albumin by those of serum globulins. Colorimetric determination of aspartate aminotransferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) activities was performed according to Reitman and Frankel [Reitman, and Frankel, 1957and Tietz 1986], respectively. Blood glucose level was determined as described by Trinder, 1969. Serum uric acid was determined according to Fossati *et al.* 1980. Serum creatinine was assayed using the method described by Fabiny and Ertingshausen 1971. The above mentioned serum biochemical parameters were assayed using reagent kits supplied by StanBio Laboratories incorporation, USA.

Tissue Specimens for Histopathological Examination

Tissue specimens including bursa, thymus and spleen were collected at weekly intervals and fixed in 10% neutral buffered formalin for preparing paraffin tissue sections at 4-6 μ thickness. These sections were stained with hematoxylin and eosin [Bancfort and Stevens, 1996].

Statistical Analysis

Values were expressed as mean \pm SD. Statistical comparisons among the means of different experimental groups were made with completely randomized two ways ANOVA "Student-Newman-Keuls test" by COSTAT program version one. A probability "P" value of <0.05 was assumed for statistical significance.

RESULTS AND DISCUSSION

Chicken Performance Results

Results of body weight, organ weight and organ weight/ body weight ratio are illustrated in tables, 1&2.

Compared to control group A, results showed increases in body weight values of both vaccinated groups B and C but, these increases were higher in group C than those observed in group

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



B. Both vaccinated groups had feed intake and FCR values more than control group but, values of group C was higher than those of group B. Lymphoid organs (liver, bursa and spleen) body weight of vaccinated groups were higher than those of control group.

Immunological Results

Mean haemagglutination inhibition (HI) antibody titers against avian influenza vaccine (H5N1) of chicken in various experimental groups at weekly intervals are summarized in table, 3.

In our study, for evaluating the effect of AI vaccines on immune system, experimental chicks were vaccinated with inactivated AI vaccine by S/C route at 9th day of age. The results showed, mean values of HI titer against avian influenza virus (H5N1) in chicks vaccinated with bivalent vaccine (AI&ND) were higher than those values of chicks vaccinated with monovalent vaccine [El Sayed et al.,2011].

Clinicopathological Findings

Erythrogram

Mean values of erythrogram [packed cell volume (PCV %), hemoglobin concentration (Hb) and erythrocytes count (RBCs)] of different experimental groups are illustrated in table, 4.

Erythrogram mean values of different experimental groups, in comparison to those of control group (A) showed, insignificant changes in vaccinated groups B and C.

Leukogram

Mean values of leukogram [total leukocyte count (TLC), neutrophil, lymphocyte and monocyte counts] of different experimental groups are illustrated in tables, 5&6.

Compared to control group, results showed significant leukocytosis due to significant lymphocytosis started from the 2nd week till the end of the experiment in both vaccinated groups.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



This lymphocytosis was a result of immunostimulatory effect of vaccination. Lymphocytosis which observed in group C (vaccinated with bivalent AI vaccine) was higher than those in group B (vaccinated with monovalent AI vaccine). Lymphocytosis is known to be occurred post vaccination [Latimer, 2011] and the avian influenza vaccines have been shown to induce antigen specific lymphocyte responses that may explain the observed lymphocytosis [Blazevic et al., 2000; Epstein, and Price, 2010 and Stephenson et al., 2010].

Serum Biochemical Evaluation

Statistical analysis of different serum biochemical parameters of different experimental groups is illustrated in tables, 7-10.

Compared to control group, protein profile results showed, no significant changes were observed in albumin concentration while, significant hyperproteinemia due to hyperglobulinemia with significant decrease in A/G ratio started from 2nd week till the end of the experiment in both vaccinated groups was recorded. This hyperglobulinemia may be attributed to the high levels of gamma globulins (immunoglobulin especially IgG and IgA) associated with chicken vaccination by AI vaccine [Mallick et al., 2011]. Hyperglobulinemia which observed in group C (vaccinated with bivalent AI vaccine) was higher than those in group B (vaccinated with monovalent AI vaccine). Activity of serum liver enzymes (AST, ALT and ALP) and concentrations of blood glucose, serum creatinine and uric acid showed insignificant changes throughout the experiment in all groups. The before mentioned results revealed that, the vaccination with both inactivated monovalent and bivalent AI vaccines did not affect liver and kidney functions.

Histopathological Findings

Bursa Histopathology

Compared to normal bursa of Fabricius structure of control group (Fig. 1a), histopathological findings revealed the presence of interfollicular congestion (Fig. 1b) and apparently normal structure of lymphoid follicles in groups B and C, respectively at the 2nd and

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



3rd week post vaccination (P.V). At 4th week post vaccination, group B showed presence of interfollicular edema (Fig. 1c), while group C revealed lymphocytic hyperplasia and lymphoblastic activation (Fig. 1d). At 5th week post vaccination, group B showed normal bursal lymphoid follicles similar to the control one, while, group C revealed severe lymphocytic hyperplasia as in (Fig. 1d).

Thymus Histopathology

In comparison to the histopathology of thymus control group section (Fig. 2a), both vaccinated groups B and C showed apparently normal cortex and medulla at 2nd week post vaccination as in control one. At 3rd week post vaccination, thymus section of group B revealed congested medulla (Fig. 2b) while, that of group C showed apparently normal histologic structure. At 4th and 5th week post vaccination, thymus section of group B showed normal histologic cortex and medulla, while group C section revealed slight lymphocytic hyperplasia (Fig. 2c).

Spleen Histopathology

Comparing to spleen histopathological section of control group (Fig. 3a), both vaccinated groups B and C at 2nd week post vaccination were more or less similar to the control one. At 3rd and 4th week post vaccination, spleen section of group B showed slight lymphocytic depletion (Fig. 3b), while those of group C were similar to the control one. At 5th week post vaccination there is no histopathological changes were detected in group B, while lymphoblastic activation were recognized in group C (Fig. 3c).

CONCLUSION

From the present study, it is concluded that, the used bivalent (H5N1&ND) AI vaccine has immunostimulatory effect higher than monovalent (H5N1) AI which reflected on increasing the immune response of its vaccinated chicken against AI vaccine which manifested by its higher globulins concentration and higher mean (HI) antibody titers of chicken against avian influenza virus (AIV) and confirmed histopathologically by the observed hyperplasia of the lymphoid organs.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



REFERENCES

Bancfort, J.D. and A. Stevens, 1996. Theory and practice of histological technique. 4 ed., New York: Churchill Livingstone.

Blazevic, V., C.M. Trubey and G.M. Shearer, 2000. Comparison of in vitro immunostimulatory potential of live and inactivated influenza viruses. *Hum. Immunol.* ;61:845–9.

Dumas, B.T. and H.G. Biggs, 1972. Standard Methods of Clinical Chemistry. Vol 7. Academic Press, New York, pp: 175

EFSA, 2008. Animal health and welfare aspects of avian influenza and the risk of its introduction into the EU poultry holdings. *EFSA J.* 715:1–161.

Epstein, S. L. and G. E. Price, 2010. Cross-protective immunity to influenza A viruses, *Expert Review of Vaccines*, 9(11): 1325–1341.

El-Sayed, D. A. A.; A. M Abdou, S. M. M. Shalash, H. M. Safaa and S. A. Riad, 2011. Productivity and immune response of broiler chickens vaccinated with different avian influenza vaccines at one or seven days of age. *Australian J. Basic and Applied Sci.*, 5(10): 325-334.

Fabiny, D.L. and G. Erttingshausen, 1971. Automated reaction-rate method for determination of serum creatinine. *Clin. Chem.*, 17: 696-700.

Feldman, B.F., J.G. Zinkl and N.C. Jain, 2000. "Schalm's Veterinary Hematology" 5 ed., Lea and Febiger, Philadelphia, U.S.A.

Fossati, P., L. Prencipe and G. Berti, 1980. Use of 3, 5-dichloro-2 hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clin. Chem.*, 26: 227-231.

Kaleta, E.F. and O. Siegmann, 1978. Kinetics of NDV specific antibodies in chickens. Analysis of frequency distributions of antibody titers against Newcastle disease virus by

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



investigation of random samples in chicken flocks. Comparative Immunol. . Microbiol. Infect. Dis., 1: 83-92.

Kilbourne, E. D., 1987. Influenza. Plenum: NY, 1—359.

Latimer, K.S., 2011. Duncan &Prasse's Veterinary Laboratory Medicine: Clinical Pathology, 5th ed., Wiley-Blackwell, chapter 2: 45-82.

Lucio, B. and S.B. Hithcner, 1980. Immunosuppression and active response induced by infectious bursal disease in chicken with passive antibodies. Avian Dis., 24: 189-196.

Mallick, A.I., P. Parvizi , L.R. Read , E. Nagy, S. Behboudi and S. Sharif, 2011. Enhancement of immunogenicity of a virosome-based avian influenza vaccine in chickens by incorporating CpG-ODN. Vaccine, 29 (8):1657-65.

OIE-Manual, 2004. Highly pathogenic avian influenza (fowl plague), in the world organization for animal health, chapter (2, 7&12), in manual of diagnostic tests and vaccines for terrestrial animals, 5th ed., Paris, France.

Reitman, S. and S. Frankel, 1957. A colorimetric method for determination of oxaloacetic transaminase and serum glutamic pyruvic transaminase. Am. J. Clin. Pathol., 28: 56-63.

Sims, L. D., T. M. Ellis, K. K. Liu, K. Dryting and H. Wong, 2003. Avian influenza in Hong Kong 1997-2000. Avian Dis., 47: 832-838.

Stephenson, I., F., A. Hayden, Osterhaus et al., 2010. Report of the fourth meeting on influenza vaccines that induce broad spectrum and long-lasting immune responses, World Health Organization and Wellcome Trust, London, United Kingdom. Vaccine, 28(23): 3875–3882.

Swaine, D. E., 2009. Avian influenza vaccines and therapies for poultry. Comp. Immunol. Microbiol. Infect. Dis. 32:351–363.

Tietz, N.W., 1986. Text Book of Clinical Chemistry. Philadelphia: WB Saunders.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Trinder, P., 1969. Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J. Clin. Pathol., 22(2): 246.

Van den Berg, T., B. Lambrecht, S. Marché, M. Steensels, S. Van Borm and M. Bublo, 2008. Influenza vaccines and vaccination strategies in birds. Comp. Immunol. Microbiol. Infect. Dis. 31:121–165.

Weichselbaum, T.E., 1946. An accurate rapid method for determination of protein in small amounts of blood, serum and plasma. Am. J. Clin. Pathol., 7: 40.

Table (1): Average body weight gain (BWG), food intake and food conversion rate (FCR) of different experimental groups

Group	Vaccine	Age / weeks	BWG Mean \pm SD	FI/ gm	Weekly FCR
A	Control	2	116.67 \pm 8.91	55.21	3.60
		3	146.25 \pm 6.24	109.32	2.79
		4	209.38 \pm 3.17	140.54	2.77
		5	221.21 \pm 4.08	148.13	2.69
Mean			173.38	113.30	2.96
B	AI	2	130.75 \pm 17.01	54.11	3.65
		3	193.98 \pm 9.23	112.20	3.70
		4	219.49 \pm 13.14	147.35	3.82
		5	240.52 \pm 12.16	154.61	4.02
Mean			196.19	117.07	3.80
C	AI + ND	2	145.52 \pm 9.88	56.20	3.69
		3	210.27 \pm 4.72	119.50	3.73
		4	282.17 \pm 4.79	152.45	3.89
		5	294.31 \pm 4.81	161.94	4.23
Mean			233.07	122.52	3.89

Group (A) represents control group (unvaccinated). Group (B) represents monovalent (H5N1) vaccinated group. Group (C) represents bivalent (H5N1&ND) vaccinated group

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

**Table (2): Average body weight, organ weight and organ/body weight ratio of different experimental groups**

Group	Vaccine	Age / ws	Body weight±SD	Liver		Bursa		Spleen	
				Weight±SD	Ratio	Weight±SD	Ratio	Weight±SD	Ratio
A	Control	2	116.67±8.91	4.36±0.29	3.74	0.55±0.02	0.47	0.21±0.03	0.18
		3	146.27±6.24	4.64±0.01	3.17	0.64±0.02	0.44	0.28±0.01	0.19
		4	209.37±3.17	6.57±0.46	3.14	0.92±0.00	0.44	0.32±0.01	0.15
		5	221.21±4.08	6.68±0.52	3.02	1.12±0.31	0.51	0.41±0.05	0.19
B	AI	2	130.95±17.01	4.24±0.42	3.24	0.59±0.04	0.45	0.27±0.06	0.21
		3	193.98±9.23	4.59±0.48	2.37	0.81±0.10	0.42	0.31±0.04	0.16
		4	219.29±13.14	6.62±0.15	3.02	1.19±0.13	0.54	0.40±0.08	0.18
		5	240.52±12.16	6.72±0.31	2.73	1.36±0.53	0.57	0.53±0.07	0.22
C	AI + ND	2	145.52±9.88	4.37±0.31	3.00	0.60±0.02	0.41	0.28±0.03	0.19
		3	210.27±4.72	4.63±0.27	2.20	0.92±0.07	0.44	0.34±0.01	0.16
		4	282.17±4.79	6.71±0.65	2.38	1.26±0.07	0.45	0.43±0.01	0.15
		5	294.31±4.81	6.84±0.73	2.32	1.51±0.12	0.51	0.65±0.09	0.22

Group (A) represents control group (unvaccinated). Group (B) represents monovalent (H5N1) vaccinated group Group (C) represents bivalent (H5N1&ND) vaccinated group

Table (3): HI antibody titers against AI and ND virus in vaccinated chicken groups

Group	Age/Days	AI- HI test								ND-HI test							
		Distribution of titre log ₂ TRN							Mean±SD	Distribution of titre log ₂ TRN							Mean±SD
		0-2	3	4	5	6	7	8		0	1	2	3	4	5	6	
A	Control	0			1	2	3		2.8±0.75					3		3	5.0±1.10
		1		1	3		2		3.1±1.12			2	1	3			4.2±0.98
		2		1	2	1	1	1	3.8±1.63			2	3	1			3.8±0.75
		3		1	3	1			4.0±0.63		1	2	2				2.2±0.84
		4	2	3	1				4.1±0.69	1	3	1					2.0±0.71
		5	2	3	1				4.3±0.61	1	3	1					2.3±0.62
B	AI	0			1	2	3		2.7±0.75					3		3	4.9±1.10
		1		1	3		2		3.0±1.12			2	1	3			4.1±0.98
		2	1	1	3				4.5±0.80			1	2	2	1		5.5±1.05
		3	3	1	2				5.1±1.38		5	1					5.8±0.41
		4	2	1	3				5.3±1.80	1	2	2	1				6.1±1.05
		5	2	1	3				5.6±1.76	1	2	2	2				6.6±1.13
C	AI +ND	0			1	2	3		2.9±0.75					3		3	5.1±1.10
		1		1	3		2		3.1±1.12			2	1	3			4.0±0.98
		2		1	3	1		1	5.7±1.60			1	2	3			5.9±0.82
		3	2	1	1	3			6.2±1.61		3	2	1				6.2±0.82
		4		1	1	3	1		6.9±0.94		3	2	1				6.7±0.82
		5		1	1	3	1		7.6±0.43		3	2	1				7.5±0.33

Group (A) represents control group (unvaccinated). Group (B) represents monovalent (H5N1) vaccinated group. Group (C) represents bivalent (H5N1&ND) vaccinated group

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

**Table (4): Erythrogram of different experimental groups (means ± SD)**

Weeks (p.i)	RBCs count ($\times 10^6/\mu\text{l}$)			PCV (%)			Hb concentration (g/dl)		
	Group (A)	Group (B)	Group (C)	Group (A)	Group (B)	Group (C)	Group (A)	Group (B)	Group (C)
0	2.12±0.51	2.07±0.78	2.18±0.58	25.18±2.61	25.03±2.34	25.61±2.38	10.74±2.67	10.32±2.71	10.62±1.72
1	2.30±0.56	2.19±0.51	2.21±0.56	25.89±2.15	25.62±1.56	25.52±2.94	11.14±1.17	11.45±1.43	11.32±1.67
2	2.32±0.40	2.27±0.48	2.30±0.44	26.65±1.43	26.69±1.87	26.27±1.13	11.36±2.15	11.49±2.47	11.73±1.66
3	2.39±0.55	2.36±0.35	2.29±0.37	27.23±2.26	27.43±2.49	27.88±1.43	11.95±2.44	11.90±1.05	11.82±1.95
4	2.43±0.78	2.61±0.68	2.48±0.32	28.70±1.45	28.91±1.42	28.32±2.54	12.73±2.65	12.45±1.63	12.51±1.36
5	2.56±0.85	2.63±0.72	2.52±0.64	30.17±1.85	30.73±1.18	30.56±2.65	13.40±1.23	13.55±2.45	13.39±1.35
LSD	0.75			3.78			2.08		

Group (A) represents control group (unvaccinated).

Group (B) represents monovalent (H5N1) vaccinated group.

Group (C) represents bivalent (H5N1&ND) vaccinated group

Table (5): Total leukocyte count (TLC) and heterophil count of different experimental groups (means ± SD)

Weeks (p.i)	TLC ($\times 10^3/\mu\text{l}$)			Heterophil count ($\times 10^3/\mu\text{l}$)		
	Group (A)	Group (B)	Group (C)	Group (A)	Group (B)	Group (C)
0	18.03±2.46	18.61±3.05	18.22±3.66	4.18±1.55	4.17±1.16	4.22±1.48
1	18.66±2.91	18.96±2.167	18.76±4.30	4.73±1.36	4.81±1.53	4.75±1.53
2	17.97±4.16	23.16±3.43	24.01±4.45	4.51±1.22	4.44±1.44	4.46±1.17
3	18.40±2.84	22.06±4.68	23.12±4.46	4.49±1.25	4.12±1.36	4.09±1.43
4	18.76±2.43	22.12±4.52	22.90±4.92	4.43±1.13	4.38±1.58	4.43±1.78
5	18.69±3.91	22.26±3.46	22.79±2.32	4.61±1.62	4.54±1.75	4.39±1.82
LSD	1.23			0.97		

Group (A) represents control group (untreated, unvaccinated).

Group (B) represents monovalent (H5N1) vaccinated group.

Group (C) represents bivalent (H5N1&ND) vaccinated group.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Table (6): Lymphocyte and monocyte counts of different experimental groups (means \pm SD)

Weeks (p.i)	Lymphocyte count ($\times 10^3/\mu\text{l}$)			Monocyte count ($\times 10^3/\mu\text{l}$)		
	Group (A)	Group (B)	Group (C)	Group (A)	Group (B)	Group (C)
0	12.72 \pm 2.12	12.75 \pm 1.45	12.81 \pm 1.65	1.25 \pm 0.11	1.18 \pm 0.16	1.26 \pm 0.15
1	12.28 \pm 2.34	13.86 \pm 1.65	13.91 \pm 1.48	1.23 \pm 0.13	1.13 \pm 0.17	1.22 \pm 0.17
2	12.36 \pm 2.53	16.13 \pm 2.34	16.99 \pm 2.46	1.24 \pm 0.15	1.19 \pm 0.12	1.23 \pm 0.18
3	12.42 \pm 1.88	15.74 \pm 1.21	16.63 \pm 2.32	1.22 \pm 0.17	1.22 \pm 0.17	1.22 \pm 0.17
4	12.31 \pm 1.93	15.90 \pm 2.34	16.48 \pm 1.66	1.24 \pm 0.25	1.27 \pm 0.25	1.24 \pm 0.16
5	12.90 \pm 1.76	15.79 \pm 1.67	16.07 \pm 1.16	1.21 \pm 0.12	1.24 \pm 0.13	1.20 \pm 0.15
LSD	2.06			0.41		

Group (A) represents control group (untreated, unvaccinated).

Group (B) represents monovalent (H5N1) vaccinated group

Group (C) represents bivalent (H5N1&ND) vaccinated group.

Table (7): Levels of serum total proteins and albumin of different experimental groups (means \pm SD)

Weeks (p.i)	Total proteins (g/dl)			Albumin (g/dl)		
	Group (A)	Group (B)	Group (C)	Group (A)	Group (B)	Group (C)
0	2.59 \pm 0.05	2.76 \pm 0.09	2.74 \pm 0.13	1.42 \pm 0.08	1.46 \pm 0.18	1.45 \pm 0.12
1	2.36 \pm 0.11	2.83 \pm 0.19	2.94 \pm 0.17	1.48 \pm 0.13	1.50 \pm 0.06	1.53 \pm 0.06
2	2.73 \pm 0.14	3.34 \pm 0.09	3.76 \pm 0.16	1.40 \pm 0.14	1.47 \pm 0.17	1.43 \pm 0.15
3	2.68 \pm 0.13	3.14 \pm 0.18	3.45 \pm 0.18	1.41 \pm 0.13	1.45 \pm 0.13	1.34 \pm 0.05
4	2.66 \pm 0.17	3.18 \pm 0.13	3.40 \pm 0.14	1.51 \pm 0.12	1.49 \pm 0.05	1.53 \pm 0.34
5	2.30 \pm 0.12	3.27 \pm 0.16	3.48 \pm 0.16	1.55 \pm 0.16	1.52 \pm 0.15	1.58 \pm 0.26
LSD	0.16			0.49		

Group (A) represents control group (untreated, unvaccinated).

Group (B) represents monovalent (H5N1) vaccinated group

Group (C) represents bivalent (H5N1&ND) vaccinated group.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Table (8): Levels of serum globulins and A/G ratio of different experimental groups (means \pm SD)

Weeks (p.i)	Globulins (g/dl)			A/G ratio		
	Group (A)	Group (B)	Group (C)	Group (A)	Group (B)	Group (C)
0	1.15 \pm 0.09	1.18 \pm 0.13	1.20 \pm 0.09	1.23 \pm 0.13	1.24 \pm 0.08	1.21 \pm 0.07
1	1.17 \pm 0.07	1.23 \pm 0.15	1.25 \pm 0.05	1.26 \pm 0.17	1.22 \pm 0.09	1.22 \pm 0.06
2	1.23 \pm 0.03	1.65 \pm 0.09	1.89 \pm 0.17	1.14 \pm 0.12	0.89 \pm 0.14	0.76 \pm 0.09
3	1.12 \pm 0.04	1.54 \pm 0.12	1.77 \pm 0.14	1.26 \pm 0.07	0.94 \pm 0.07	0.76 \pm 0.06
4	1.12 \pm 0.06	1.64 \pm 0.15	1.78 \pm 0.18	1.35 \pm 0.15	0.91 \pm 0.05	0.86 \pm 0.13
5	1.20 \pm 0.05	1.63 \pm 0.17	1.86 \pm 0.13	1.29 \pm 0.16	0.93 \pm 0.07	0.85 \pm 0.14
LSD	0.11			0.11		

Group (A) represents control group (untreated, unvaccinated).

Group (B) represents monovalent (H5N1) vaccinated group.

Group (C) represents bivalent (H5N1&ND) vaccinated group.

Table (9): Activity of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of different experimental groups (means \pm SD)

Weeks (p.i)	AST (U/L)			ALT (U/L)			ALP (U/L)		
	Group (A)	Group (B)	Group (C)	Group (A)	Group (B)	Group (C)	Group (A)	Group (B)	Group (C)
0	152.58 \pm 11.33	153.64 \pm 13.41	152.08 \pm 11.67	31.25 \pm 1.22	31.53 \pm 1.69	30.73 \pm 1.52	133.14 \pm 10.86	132.69 \pm 11.68	133.09 \pm 9.95
1	153.91 \pm 13.15	154.42 \pm 11.51	153.67 \pm 12.74	30.47 \pm 1.13	30.11 \pm 2.57	31.05 \pm 3.53	134.11 \pm 8.93	133.06 \pm 7.87	135.51 \pm 6.27
2	154.61 \pm 13.43	155.48 \pm 12.36	154.46 \pm 13.14	31.18 \pm 1.74	30.95 \pm 2.06	30.88 \pm 4.06	133.92 \pm 11.35	133.20 \pm 9.74	137.17 \pm 8.22
3	155.19 \pm 11.75	154.16 \pm 12.77	151.18 \pm 12.75	30.62 \pm 1.57	31.61 \pm 2.11	30.98 \pm 5.75	134.13 \pm 8.55	135.32 \pm 9.26	136.40 \pm 9.53
4	156.62 \pm 12.13	150.24 \pm 11.31	154.42 \pm 11.45	31.48 \pm 1.67	30.67 \pm 2.25	30.93 \pm 3.35	135.02 \pm 9.63	134.81 \pm 7.68	133.92 \pm 9.04
5	155.28 \pm 13.45	152.23 \pm 12.64	154.58 \pm 13.06	30.99 \pm 1.38	31.19 \pm 1.65	30.79 \pm 3.24	133.94 \pm 10.48	135.47 \pm 9.51	134.86 \pm 6.98
LSD	14.69			2.98			15.55		

Group (A) represents control group (untreated, unvaccinated).

Group (B) represents monovalent (H5N1) vaccinated group.

Group (C) represents bivalent (H5N1&ND) vaccinated group.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

Table (10): Values of serum glucose, creatinine and uric acid of different experimental groups (means \pm SD)

Weeks (p.i)	Glucose (mg/dl)			Creatinine (mg/dl)			Uric acid (mg/dl)		
	Group (A)	Group (B)	Group (C)	Group (A)	Group (B)	Group (C)	Group (A)	Group (B)	Group (C)
0	240.82 \pm 7.70	242.13 \pm 12.60	244.92 \pm 9.61	0.31 \pm 0.08	0.30 \pm 0.07	0.32 \pm 0.08	5.83 \pm 0.53	5.88 \pm 0.62	5.82 \pm 0.31
1	241.05 \pm 10.17	243.09 \pm 9.53	244.85 \pm 9.74	0.33 \pm 0.05	0.29 \pm 0.05	0.31 \pm 0.07	6.22 \pm 0.54	6.14 \pm 0.61	6.35 \pm 0.33
2	244.87 \pm 12.83	242.51 \pm 15.61	249.39 \pm 7.91	0.32 \pm 0.06	0.31 \pm 0.07	0.30 \pm 0.02	5.89 \pm 0.35	5.96 \pm 0.65	5.85 \pm 0.80
3	247.16 \pm 9.21	252.73 \pm 13.64	247.68 \pm 8.64	0.34 \pm 0.09	0.29 \pm 0.04	0.33 \pm 0.04	5.91 \pm 0.57	5.87 \pm 0.67	5.90 \pm 0.43
4	251.42 \pm 9.25	247.81 \pm 10.28	250.90 \pm 8.61	0.31 \pm 0.03	0.33 \pm 0.02	0.32 \pm 0.05	6.03 \pm 0.44	5.89 \pm 0.60	6.08 \pm 1.14
5	244.96 \pm 10.27	244.39 \pm 12.77	252.49 \pm 8.79	0.33 \pm 0.07	0.32 \pm 0.03	0.34 \pm 0.07	6.51 \pm 0.42	6.62 \pm 0.54	6.37 \pm 1.09
LSD	11.03			0.14			1.42		

Group (A) represents control group (untreated, unvaccinated).

Group (B) represents monovalent (H5N1) vaccinated group.

Group (C) represents bivalent (H5N1&ND) vaccinated group.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Figure (1): Bursa of Fabricius sections of different experimental groups

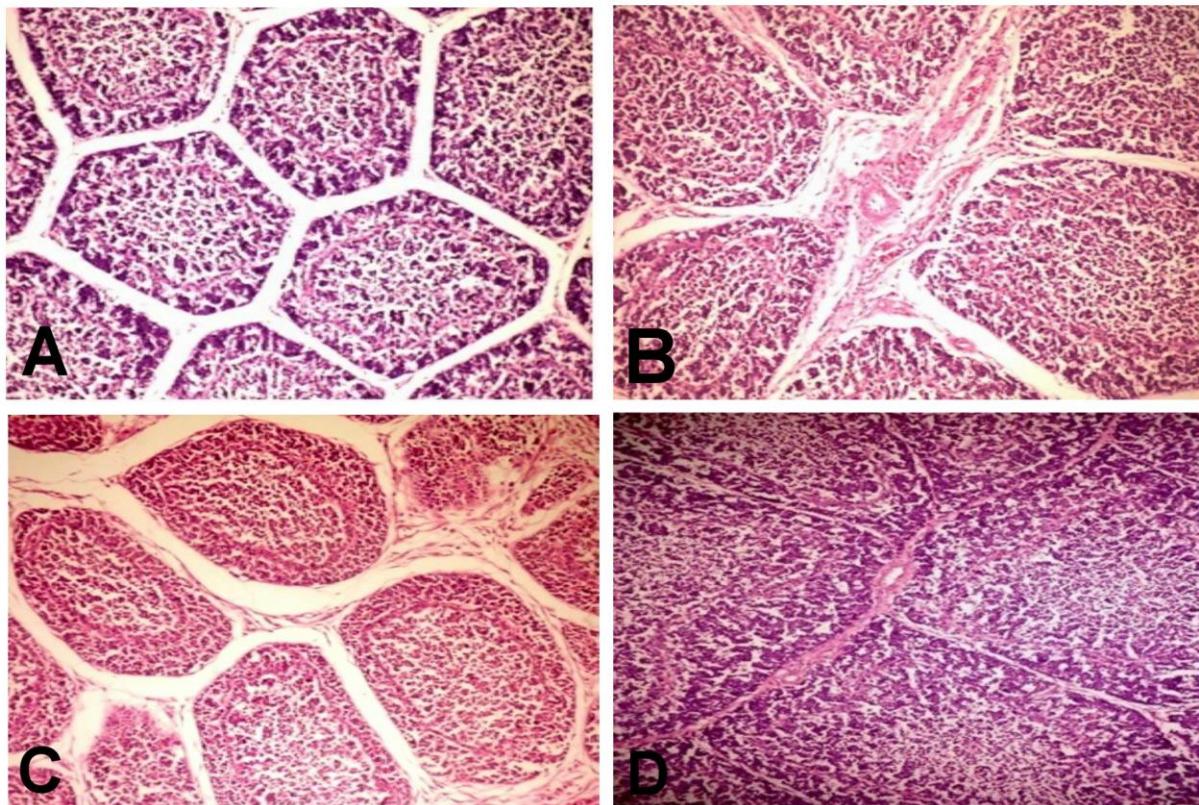


Fig. 1: (A): Bursa of Fabricius of control group A showing apparently normal lymphoid follicles (H&E X 200).

(B): Bursa of Fabricius of group B at the 2nd and 3rd week post vaccination showing interfollicular congestion (H&E X 200).

(C): Bursa of Fabricius of group B at 4th week post vaccination showing interfollicular edema (H&E X 200).

(D): Bursa of Fabricius of group C at 4th week post vaccination showing lymphocytic hyperplasia and lymphoblastic activation (H&E X 200).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Fig (2): Thymus sections of different experimental groups

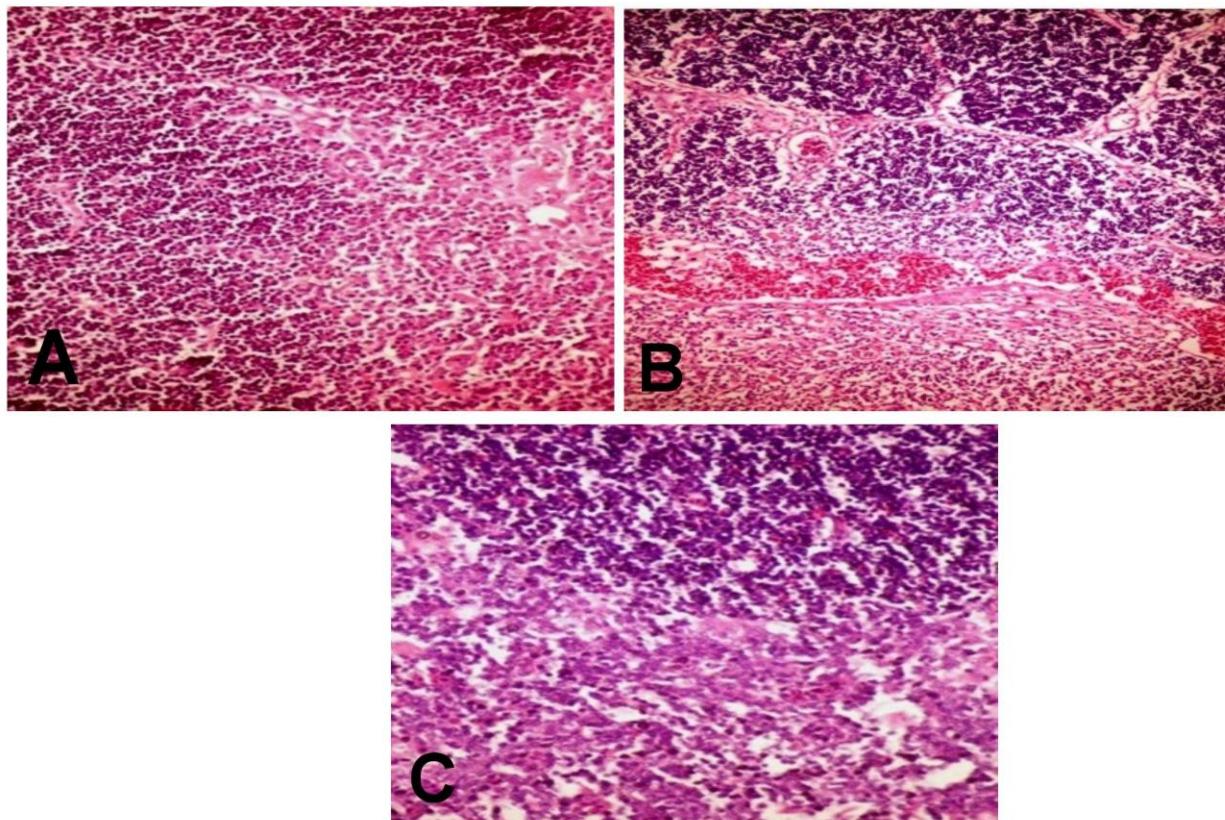


Fig. 2: (A): Thymus of control group A showing apparently normal cortex and medulla (H&E X 200).

(B):Thymus of group B at 3rd week post vaccination showing congested medulla (H&E X 200).

(C): Thymus of group C at 4th and 5th week post vaccination showing slight lymphocytic hyperplasia (H&E X 200).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Fig (3): Spleen sections of different experimental groups

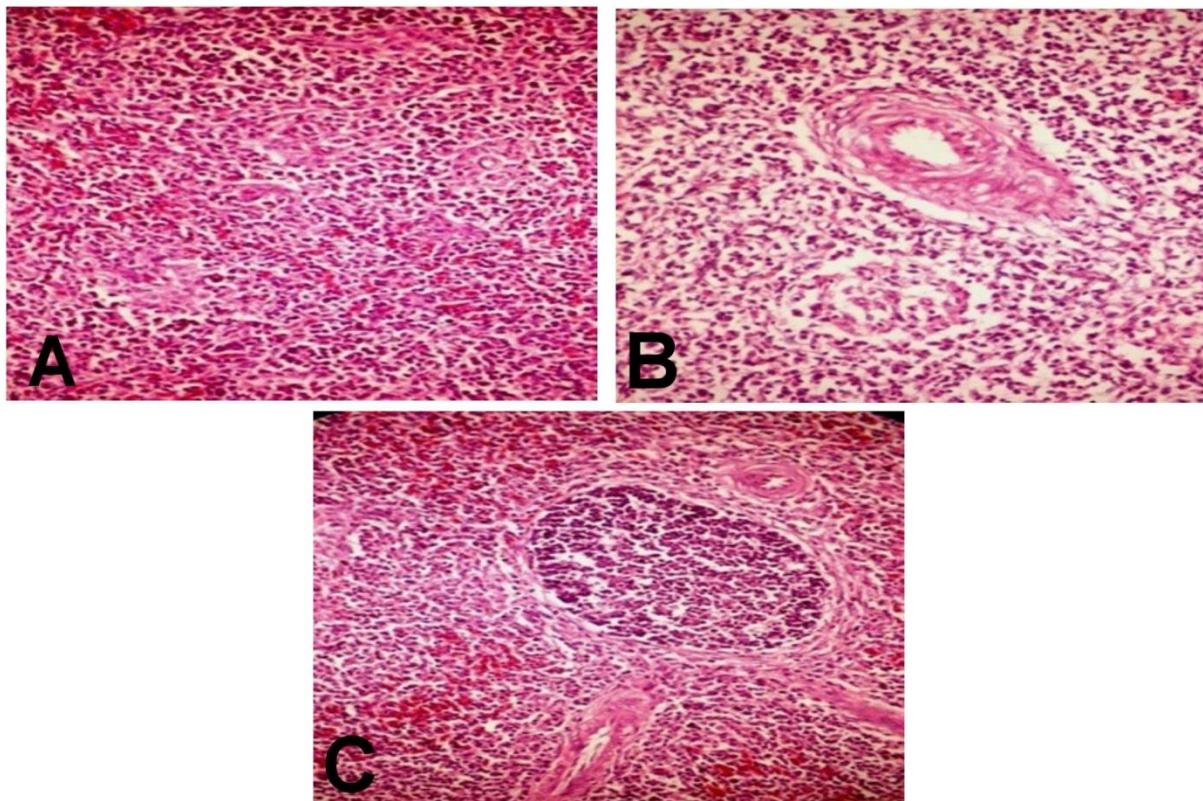


Fig. 3: (A): Spleen of control group A showing normal histological findings (H&E X 200).
(B): Spleen of group B at 3rd and 4th week post vaccination showing slight lymphocytic depletion (H&E X 200).
(C): Spleen of group C at 5th week post vaccination showing lymphoblastic activation (H&E X 200).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



CLINICAL, SEROLOGICAL AND PATHOLOGICAL CHARACTERIZATION OF RABBIT HAEMORRHAGIC DISEASE IN THE SULTANATE OF OMAN

***Mohamed S. Ahmed.^{1,2)}, Body M.²⁾, AL-Rawahi A. H.²⁾, Al-Mawaly, M.²⁾ and Al-Habsy,
S.²⁾**

¹Department of Pathology, Faculty of Veterinary Medicine, Kafr El-Sheikh University, Kafr El-Sheikh, 33516, Egypt.

²Veterinary Research Centre, Ministry of Agriculture and Fisheries, A'seeb. P.O.Box:50-Postal code: 121, Sultanate of Oman.

ABSTRACT

This study describes an outbreak of Rabbit Hemorrhagic Disease (RHD) in domestic rabbits in Oman. The provisional diagnosis was reached by history, clinical signs, postmortem and histopathological findings and experimental inoculation in adult and juvenile *albino* laboratory rabbits. The presence of etiologic agent in the liver homogenate from spontaneous and experimentally infected rabbits was demonstrated by postmortem and histopathological changes as well as haemagglutination test. The virus did not grow on embryonated chicken eggs. As far as could be ascertained, this is the first document on RHD in the Sultanate of Oman and 3rd record of the disease in Arabian Peninsula over a decade period of time.

Keywords: Rabbit, Rabbit Hemorrhagic Disease, haemorrhage, epistaxis, necrosis.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



INTRODUCTION

Rabbit Hemorrhagic Disease (RHD) also known as viral hemorrhagic disease of rabbits is rapidly fatal, with a mortality rate ranging from, 70%–100% in adult rabbits (OIE, 2010); while, young rabbits (kits) are unaffected or subclinically infected (**Mikami, et al., 1999 and OIE, 2010**). It is caused by Rabbit Hemorrhagic Disease Virus (RHDV), a member of the genus Lagovirus of family Caliciviridae (**White, et al., 2004 and Bergin, et al., 2009**). RHDV is not cultivatable in cell culture; therefore, detection of virus genome, virions, and anti-RHDV antibodies in addition to experimental infection of rabbits are required for diagnosis and virus characterization (**Strive, et al., 2009 and OIE, 2010**). The clinical manifestations of RHD have been described in different forms. The peracute form affects highly susceptible rabbits which have not been infected previously (**Xu and Chen, 1989, Anonymous, 2003 and Campagnolo, et al, 2003**). The acute form is highly prevalent in epidemic areas, affecting adults or young rabbits over the age of two months; meanwhile, most animals affected with the subacute form survive and become resistant to reinfection, but chronic form is considered rare and symptomless and the subclinical form is only hypothesised in suckling rabbits (**Xu, 1991 and Forrester, et al., 2007**). The incubation period is 1-2 days (**Reyes, et al., 1990 and Xi, et al., 1990**) or a maximum of 3 days, and death may take place 12-48 hours after sudden onset of various inconstant signs (**Moss, et al., 2002**).

The first known outbreak occurred in China in 1984 (**Liu, et al., 1984**). The disease was spread by imported Angora rabbits and killed 14 million domesticated rabbits within nine months (**Liu, et al., 1984**). By the late 1990s, outbreaks had been reported from forty countries, and rabbit hemorrhagic disease had become endemic in wild rabbit populations in Europe, Australia and New Zealand (**Cooke, 2002**). Other parts of the world including the Americas have experienced periodic outbreaks in domesticated rabbits. Wild rabbits which inhabit North America however, are not susceptible to the disease (**OIE, 2012**).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



The disease was first seen in Saudi Arabia in 1997 (**Abu-Elzein & Al-Afaleq, 1999**) and emerged in Bahrain in 2001 (**Abubakar, et al., 2005**).

MATERIALS AND METHODS

History and clinical signs: An outbreak of the disease occurred in march, 2012 in adult domestic rabbits(*Oryctologus cuniculus*) which has affected farms in the North-Eastern region (Al-Dakhaliya) of Oman. Onset of the disease was sudden with 100% morbidity and mortality rates. Death occurred in few hours to 3 days after the appearance of the clinical signs which included dullness, mouth and nostril bleeding, anorexia, gasping, and lateral paralysis. Antibiotic and supportive treatments were not effective.

Postmortem examination: Gross examination revealed the existence of blood-tinged discharge in 6 out of 52 freshly dead rabbits. Dead animals had good body condition and showed hemorrhages in subcutaneous tissue, trachea, lung, pericardium and kidneys. Enlarged and hemorrhagic liver with Splenomegaly were consistent features in all carcasses. Bacteriological examination of lung and liver tissues was insignificant. Mice inoculation tests for feed intoxication and clostridial toxins were negative.

Experimental infection: Thirty albino rabbits (*Oryctolagus cuniculus*) comprising 20 adult and 10 immature rabbits were randomly divided into 2 (equal) groups, A and B, each containing 10 adult and 5 immature animals. Each rabbit of group A received 100 µl of liver homogenate intranasally, while rabbits in group B were inoculated with equal dose of Phosphate Buffered Saline [PBS] (pH 7.2) thorough the same route and served as control. As the suspected disease is highly contagious, all sanitary and hygienic procedures were undertaken to avoid cross-infection. Animal groups were kept in secure isolated boxes till the termination of the experiment.

Sample collection: Specimens from liver, lung, spleen and heart of the naturally infected dead and the experimentally inoculated rabbits were preserved in 10% buffered formalin for histopathological examination (**Bancroft and Stevens, 1996**). Liver tissue samples were collected in sterile jars and preserved at -40 °C for further investigations.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Agglutination studies: Haemagglutination (HA) test was carried out in microtiter plate by 2 fold serial dilution of liver homogenates supernatant (10%) in PBS pH 7.2. An equal volume of 1% human of group O red blood cells was used. Ten percent healthy liver homogenates in PBS pH 7.2 (prepared from normal rabbits) served as negative control in HA tests. The plates were incubated and read according to **Abu-Elzein & Al-Afaleq, 1999** and **Robinson et al., 2002**.

Chicken embryonated egg inoculation: Ten days old chicken embryonated eggs (n = 10) were inoculated with liver homogenates with 0.1ul of liver through intra-allantoic route. Control eggs of equal number were inoculated with sterile PBS of pH 7.2. Inoculated eggs were incubated at 37 °C for 3 days and candling was performed daily during incubation. At the end of incubation, allantoic fluids were harvested and tested for haemagglutination activity (**Chasey, 1997**).

RESULTS

Naturally infected rabbits: Onset of the disease was sudden with 100% morbidity and mortality rates. Death occurred in few hours to 3 days after the appearance of the clinical signs which included dullness, mouth and nostril bleeding, anorexia, gasping, and lateral paralysis. Antibiotic and supportive treatments were not effective.

Gross examination revealed the existence of blood-tinged discharge in 6 out of 52 freshly dead rabbits. Dead animals had good body condition and showed hemorrhages in subcutaneous tissue, trachea, lung, pericardium and kidneys. Enlarged and hemorrhagic liver with Splenomegaly were consistent features in all carcasses.

Experimentally infected rabbits: A sudden death was registered in 70% (7/10) of experimentally infected adult rabbits in group A at 24 hours post inoculation, whilst 30% (3/10) culminated in the death at 48 hours post inoculation. Of these, only two rabbits exhibited epistaxis at the time of death (Fig. 1,2). Postmortem picture of experimentally infected rabbits was similar with little variation in severity of lesions to the rabbits with natural infection. The most prominent lesions found at necropsy were petechial hemorrhages in almost all organs especially in the subcutis of

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



all rabbits (Fig. 3). The livers were pale yellow or greyish, with a finely granular surface (Fig. 4). The tracheal mucosa was hyperemic and contained abundant frothy fluids. The lung in all animals was hyperemic and edematous with multifocal haemorrhages up to one cm or more in diameter (Fig. 5,6). Splenomegaly with severe hyperemia (Fig. 7) and petechial haemorrhages in the epicardium (Fig. 8) were also noticed. All immature rabbits in group-A survived for one week post inoculation and showed no lesions on autopsy.

Microscopically, the livers showed multifocal necrosis and leukocytic infiltration, small, scattered intralobular foci of haemorrhage (Fig. 9). Foci of disseminated necrosis became confluent and were associated with mild to moderate inflammatory infiltrate in portal spaces and sinusoids and forming extensive local areas with mild to moderate inflammatory infiltrate in portal spaces and sinusoids (Fig. 10). Other hepatocytic lesions were bile pigment and/or extensive deposition of iron pigment (Fig. 11). Tracheal and pulmonary lesions were mainly of the hyperaemic-oedematous type, often associated with haemorrhages (Figs. 12). There was lymphoid depletion and haemorrhages in the lymphoid tissues (Fig. 13) and the sub-epicardial haemorrhages in the heart (Fig. 14).

Haemagglutination titers in the liver homogenates from spontaneously and experimentally infected adult rabbits ranged in the middle of 1/512 to 1/2048. This activity was inhibited by hyperimmune serum. Allantoic fluids harvested from inoculated embryonated eggs did not show any haemagglutination activity.

Inoculated chicken embryos remained viable till the end of the experiment (3-day duration) without any gross pathological change.

DISCUSSION

In the present outbreak the affected rabbits were found dead without any clinical manifestation, nonetheless, hematuria and blood stained foamy discharge from the nostrils were occasionally observed which similar to the peracute form of RHD that could be due to first ever

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



introduction of the virus in the region (**Muller, et al., 2009, Abu-Elzein & Al-Afaleq, 1999 and Abubakr, et al., 2005**).

In the present study, adult rabbits inoculated with liver homogenate exhibited typical clinical pattern of the disease (incubation period, and percent mortality), postmortem and microscopical lesions, while young rabbits did not develop the disease. The age-related resistance in very young rabbits is still poorly understood, but maternal antibodies have been suggested as one possible explanation for survival in older kittens (**OIE, 2007**) or attributed to low susceptibility of hepatocytes (**Mikami, 1999**). Naïve rabbits (4-10 weeks old) born of RHD antibodies free dams were impervious to experimental infection at 4-week, however, susceptibility increased rapidly thereafter. Acquisition of hepatic function capable of eliciting pathogenic potential of the virus starts at 6 week of age (**Morissey, et al., 1991**).

Reports on the rabbit hemorrhagic disease are scarce, especially on the gross and histopathological aspects. Lesions are likely to be caused by viraemia; sudden death is suggested to be a consequence of multiple organ failure resulting from lung oedema and haemorrhage, circulatory disorders and hepatic necrosis (**Xu & Chen, 1989 and Uyttenbroek, et al., 1990**). We hypothesised that the necrotic and inflammatory damage of the liver is central to the pathogenesis of the disease due to viral replication within hepatocytes. Prominent virus-induced hepatic damage is supported by the demonstration that the concentration of the virus is highest in the liver (**Mori, et al., 1981 and Patton, 1989**). Antigen-antibody reaction is known to effectively provoke microthrombosis and subsequent hepatic necrosis (**Marcato, et al., 1989, Xu & Chen, 1989 and Marcato, et al., 1991**). Hemorrhagic lesions throughout the body have been ascribed to disseminated intravascular coagulopathy (**Marcato, et al., 1991**) leading to poor blood coagulation and multifocal haemorrhages. Disseminated intravascular coagulation (DIC) may result from systemic endothelial damage caused by viraemia, but it could also be a consequence of massive hepatic necrosis (**Patton, 1989**) which might have augmented the hepatic lesions in the rabbits of the present report

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Efforts on more than 40 primary and continuous cell cultures failed to support viral propagation; nevertheless, Chinese workers (**Ji, et al., 1991**) have successfully adapted the virus on DJ rabbit kidney cell strain. Historically, all attempts to infect laboratory animals and chicken embryos were also abortive. Experimental production of the disease in target species is till foremost laboratory method for viral replication (**Le Gall-Reculé, et al., 2011**). Consistent with the forementioned report, failure of propagation of the virus on the embryonated chicken eggs was also observed in present study which demonstrated by non-hemagglutinating activity of allantoic fluid.

In the present work, source and mode of inflowing of infection in the country could not be track down. However, importation of rabbit meat from disease-endemic-countries might have played role in introducing the virus in the Sultanate. Furthermore, source of infection from contiguous Arab countries where the disease has been recorded during last decade, cannot be overlooked.

CONCLUSION:

Several features optimized the outbreaks described in the present work as RHDV infection for all intent and purposes including (i) experimental production of the disease in the adult rabbit (ii) postmortem and histopathological lesions in the naturally and experimentally infected rabbits (iii) inability of the virus to grow on chicken embryonated eggs and (iv) agglutinating activity of liver homogenate against human type O red blood cells from naturally and experimentally infected rabbits. As far as could be ascertained the occurrence of rabbit hemorrhagic disease has not heretofore been reported from Sultanate of Oman.

To the best of authors' knowledge, the present study is the first account on the occurrence of rabbit viral hemorrhagic disease in the Sultanate of Oman and the third report in the Arabian Peninsula.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



ACKNOWLEDGEMENTS: The authors are thankful to the Director General of Agricultural and Livestock Research, Sultanate of Oman and staff of Veterinary Research Centre for their cooperation and support during this work.

REFERENCES

- Abubakar, M.I., Gould, E.A. Fadlalla, M.E. & Abuobeida, S.A. (2005).** Rabbit hemorrhagic disease in Bahrain. *Revue Elev. Vet. Pays Trop.* 58: 217-219.
- Abu-Elzein, E.M. & Al-Afaleq, A.I. (1999).** Rabbit Hemorrhagic disease in Saudi Arabia. *Vet. Rec.* 144: 480-481.
- Anonymous, (2003).** Rabbit Hemorrhagic disease. The Center for Food Security and Public Health. College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA.
- Bancroft, J. D. and Stevens, A. (1996).** Theory and Practice of Histopathological Techniques. Fourth edition. 111-129.
- Bergin, I.L., Wise, A.G., Bolin, S.R. et al. (2009).** Novel calicivirus identified in rabbits, Michigan, USA. *Emerg Infect Dis.* 15: 1955–1962.
- Campagnolo, E., Ernst, M., Berninger, M. et al. (2003).** Outbreak of rabbit hemorrhagic disease in domestic lagomorphs. *J Am Vet Med Assoc.* 223:1151-1155, 1128.
- Chasey, D (1997).** Rabbit haemorrhagic disease: the new scourge of *Oryctolagus cuniculus* Lab. Anim. 31: 33-44
- Cooke, B. D. (2002).** Rabbit hemorrhagic disease: field epidemiology and management of wild rabbits population. *Rev. Sci. Tech. Off. Int. Epiz.* 21: 347-358.
- Forrester, N.L., Trout, R.C., Gould, E.A. (2007).** Benign circulation of rabbit haemorrhagic disease virus on Lambay Island, Eire. *Virol.* 358: 18-22.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Ji, C.Y., Du, N.X. & Xu, W.Y. (1991). Adaptation of the viral hemorrhagic disease virus of rabbits to the DJRK cell strains. *Rev. Sci. tech Off. Int. Epiz.* 10: 337-345.

Le Gall-Reculé, G., Zwingelstein, F., Boucher, S. et al. (2011). Detection of a new variant of rabbit haemorrhagic disease virus in France. *Vet Rec.* 168:137–138.

Liu, S.J., Xue, H.P., Pu, B.Q. & Quian, N.H. (1984). A new viral disease in rabbits (in Chinese). *Anim. Hus. vet. Med.*, 16 (6): 253-255.

Marcato, P.S., Benazzi, C., Galeotti, M. and Della Salda, L. (1989). L'epatit necrotica infettiva dei leporidi. Nuove ricerche sulla patogenesi della malattia emorragica del coniglio e della lepre. *Riv. Coniglicoltura.* 26(8): 41-50.

Marcato, P.S., Benazzi, C. Vecchi, G. et al. (1991). Clinical and pathological features of viral hemorrhagic disease of rabbits ad European brown hare syndrome. *Rev. Sci. Tech. Off. Epiz.* 10: 371-392.

Mikami O, Kimura T, Ochiai K, Itakura C (1999). Hepatic lesions in young rabbits experimentally infected with rabbit haemorrhagic disease virus. *Res Vet Sci.* 66: 237–242.

Mori, W., Aoki, N. and Shiga, J. (1981). Acute hepatic cell necrosis experimentally produced by viral agents in rabbits. *Am. J. Pathol.* 103: 31-38.

Morissey, J.P., Le Gall, G., & Boilletot, E. (1991). Hepatitis of viral origin in Leporidae: introduction and etiological hypotheses. *Rev. Sci. tech Off. Int. Epiz.* 10: 283-295.

Moss, S.R., Turner, S.L. Trout, R.C. et al. (2002). Molecular epidemiology of rabbit hemorrhagic disease. *J. Gen. Virol.* 83: 2461-2467.

Muller, A., Freitas, J., Silva, E., et al. (2009). Evolution of rabbit haemorrhagic disease virus (RHDV) in the European rabbit (*Oryctolagus cuniculus*) from the Iberian Peninsula. *Vet Microbiol.* 135: 368–373.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Patton, N.W. (1989). Viral haemorrhagic disease of rabbit. *J. appl. Rabbit Res.*, 12 (1): 64-66.

Reyes, G.R., Purdy, M.A., Kim, J.P., et al., (1990). Isolation of a cDNA from the virus responsible for enterically-transmitted non-A non-B hepatitis. *Science*. 247: 1335-1339.

Robinson, A.J., Kirkland, D., Forr, R.I. et al., (2002). Serological evidence for the presence of a calicivirus in Australian wild rabbits, *Oryctolagus cuniculus*, before the introduction of RHDV: its potential influence on the specificity of a competitive ELISA for RHDV. *Wildl. Res.* 29: 655–662.

Strive, T., Wright, J. & Robinson, A. (2009). Identification and partial characterisation of a new Lagovirus in Australian wild rabbits. *Virol.* 384: 97–105.

Uyttenbroek, E., Nauwinck, H., Ducatelle, R. et al. (1990). Pathology and aetiology of natural and experimental European brown hare. *Schweizer Arch. Tierheilk.* 132: 478-479.

White, P.J., Trout, R.C., Moss, S.R. et al. (2004). Epidemiology of rabbit haemorrhagic disease virus in the United Kingdom: evidence for seasonal transmission by both virulent and a virulent modes of infection. *Epidemiol. Infect.* 132: 555–567.

World Organization for Animal Health [OIE] (2007). Handisstatus II [database online]. OIE; 2007. Available at: <http://www.oie.int/hs2/report.asp?lang=en>.

World Organisation for Animal Health [OIE] (2010). Terrestrial manual. Chapter 2.6.2. Rabbit haemorrhagic disease. 2010 [cited 2012 Feb 27]. http://www.oie.int/fi/leadmin/Home/eng/Health_standards/tahm/2.06.02_RHD.pdf.

World Organisation for Animal Health [OIE] (2012). Variant Rabbit Hemorrhagic Disease Virus in Young Rabbits, Spain Kevin P. Dalton, Inés Nicieza, Ana Balseiro, María A. Muguerza, Joan M. Rosell, Rosa Casais, Ángel L. Álvarez, and Francisco Parra. Emerging Infectious Diseases • www.cdc.gov/eid. 18(12): 2009-2012.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Xi J., Graham, D.J., Wang, K. & Estes, M.K. (1990). Norwalk virus genome cloning and characterization. *Science*. 250: 1580-1583.

Xu, W.Y., (1991). Viral hemorrhagic disease of rabbits in the People's of Republic of China: epidemiology and virus characterization. *Rev. Sci. Tech. Off. Epiz.*, 10: 393-408.

Xu, Z.J., Chen, W.X. (1989). Viral hemorrhagic disease in rabbits: a review. *Vet Res. Comm.*, 13: 205-212.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Fig. 1. Epistaxis in naturally infected rabbits
rabbits (black arrow)



Fig. 2. Epistaxis in experimentally infected
(black arrow)



Fig. 3. Haemorrhages in the subcutis
(white arrows)



Fig. 4. Multiple necrotic areas on liver
(white arrows)

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Fig. 5. Trachea filled with frothy blood hemorrhages

(white arrow)



Fig. 6. Edematous lungs with multifocal

(white arrows)



Fig. 7. Splenomegaly with severe congestion

(white arrow)

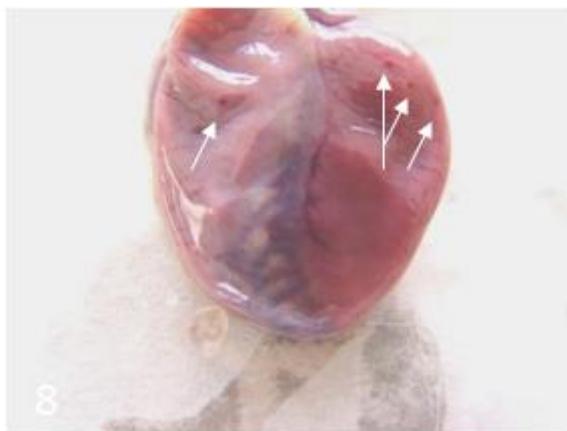


Fig. 8. Petechial epicardial hemorrhages

(white arrows)

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

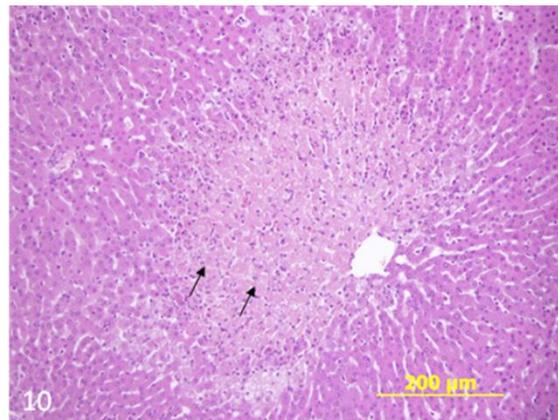
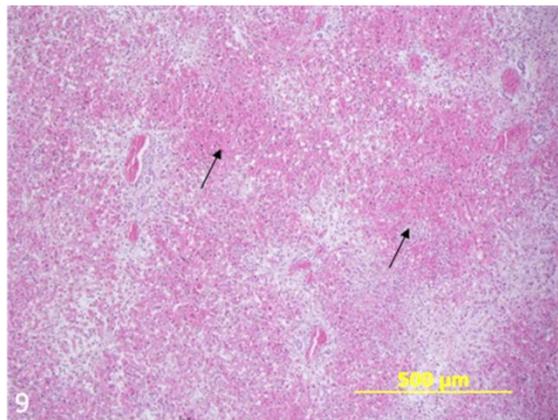


Fig. 9. Massive haemorrhages in the liver (arrows)

Fig. 10. Necrosis of hepatocytes and
Infiltration of mononuclear cells
(arrows)

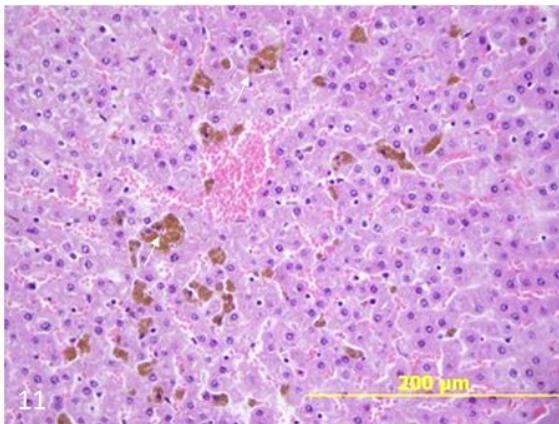


Fig. 11. Brown iron pigments in the hepatocytes

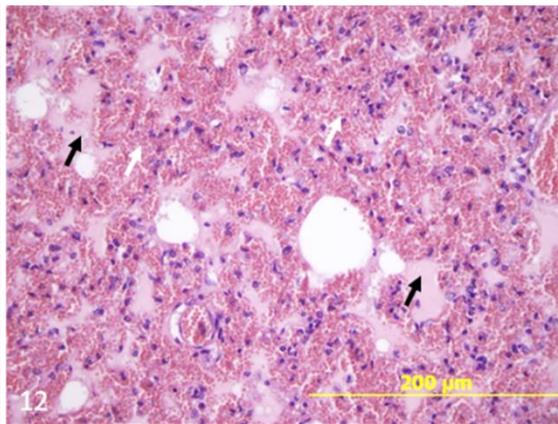


Fig. 12. Pulmonary haemorrhages (white arrows) and alveolar oedema (black arrows)

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

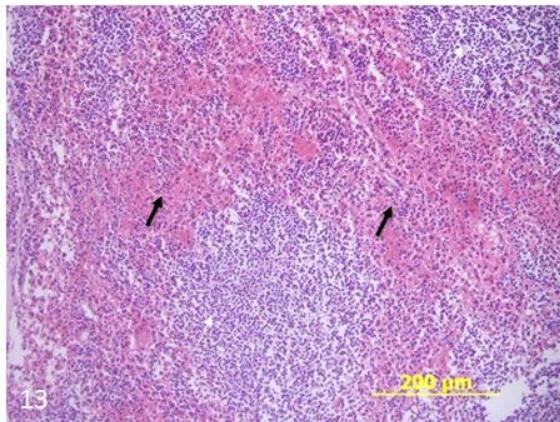


Fig. 13. Lymphoid follicle depletion (white arrows) and medullary haemorrhages (black arrows) in the spleen

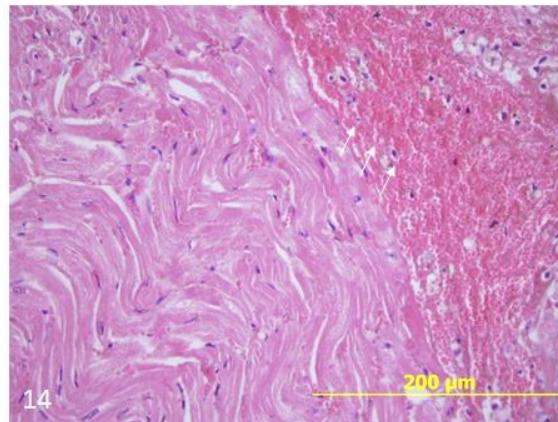


Fig. 14. Subepicardial haemorrhages (arrows)

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



PATHOLOGICAL EVIDENCE FOR A FATAL NERVOUS DISEASE IN GROUPER FISH IN LIBYA

¹Sayed R. Al-Attar, ²Mahir Kubba, and ³Jameela T. Rizgalla

1-Department of Pathology ,Faculty of Vet. Med. Zagazig University ,Egypt.

2- Department of Pathology ,Faculty of Vet. Med. Tripoli University. Libya.

3-Department of Aquiculture ,Faculty of Agriculture, Tripoli University, Libya

ABSTRACT

Huge Grouper fish mortalities at the East Libyan cost during 2011 were intensively investigated. Clinical, gross and histopathologic examinations were carried out in addition to bacterial isolation and parasitic detection. Microscopic tissue examination revealed different lesions in various organs. Of peculiar importance were those in the brain and eyes. The brain tissue showed congestion and edema in Virchaw Robin space with perivascular cuffing, multiple stroma vacuolation (malacia). Neuronal degeneration, vacuolation and necrosis were evident and associated with gliosis and lymphocytic aggregations. The eyes were congested and had hemorrhages and telangiectasis in the sclera and retina, lens degeneration and calcification along with corneal ulcers and hemorrhages. Such tissue changes were similar to those reported by many authors to be associated with viral nervous necrosis in different kinds of fish including Groupers. Though unconfirmed locally, adopting such etiological factor in causing this periodic massive mortalities could be of worth consideration.

Key words: Grouper fish mortality, fatal nervous sign, Malacia, Gliosis, Telangiectasis

INTRODUCTION

Grouper fish belong to Serranidae and subfamily Epinephelinae which is widely distributed in the tropical and subtropical regions. It is of great economic value and constitutes a major component of the coastal fishery wealth in Asia. It lives freely as well as in cultures which make

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



up an essential source in fish industry. At least 21 species of groupers are cultured in Asia (**Leong, 1994**).

A major production constraint in grouper culture is heavy mortality due to diseases as well\as stress during collection and transportation. (**Erlinda, 1996**). Diseases of cultured grouper may be due to infectious agents including viruses, bacteria, fungi and parasites. Non-infectious disease agents include nutritional imbalances and environmental factors may also contributing in the disease process. Bacteria are very common in the aquatic environment. Most bacterial disease agents are part of the normal flora of the water. They cause disease only when the fish are stressed due to poor environmental conditions. The pathogenic bacteria that have been identified were related to Vibrio sp., Aeromonas sp., pasteurella sp., and Streptococcus sp. (**Hartono et al. 2000**).

A large number of parasites which infest the exposed parts of the body or the internal organs have also been reported. These include protozoans, myxozoans, microsporans, monogeneans, trematodes, crustaceans, nematodes, cestodes, acanthocephalans and hirudineans (**Nagasaki, 2004**).

Among viral causes of disease, Betanodaviruses are responsible for disease commonly called "viral nervous necrosis(VNN)"or "viral encephalopathy and retinopathy(VTR)" which is the most contagious diseases reported in many marine fish species including groupers (**Arimoto et al. 1993**). Laboratory studies have shown that Betanodavirus can spread vertically (**Kai et al. 2010**), horizontally or by feeding of contaminated live food or raw contaminated fish (**Gomez et al. 2010**). Currently, there are four species of betanodavirus based on genetic analysis (**ICTV 2009**). These viral species specifically infect certain types of fish but different species of betanodavirus may infect a number of other fish species. One species, the Red spotted grouper nervous necrosis virus (RGNNV) specifically infect Red spotted grouper and has a favorable temperature of infection ranging from 25-30°C (**Hata et al. 2007**). Clinical signs reflect nervous system involvement which shows abnormal swimming behavior including vertical positioning and spinning, flexing of the body and muscle tremors which may cause traumatic injuries. There

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



is hyperinflation of the swim bladder which keep the affected fish at the surface (**Bovo and Florio 2008**). These viruses damage the central nervous system in susceptible fish species with vacuolation and necrosis in the brain, spinal cord and retina of the eye. Younger stages of fish are typically affected although older market-size fish can be affected as well, with losses ranging from 15-100% (**Yanong, 2010**). The objective of our study is to explore the possibility of viral nervous necrosis(VNN)" or "viral encephalopathy and retinopathy(VTR) as a probable cause for the major mortalities of grouper fish the in the East Libyan cost during 2011.

MATERIALS AND METHODS

Thirty grouper fish of different ages were collected from different area along the East costal region from Tobrok to El-bordy in Libya and from Marsa Matroh in Egypt during 2011. 20 samples were clinically diseased and 10 samples were apparently healthy. Post mortem examination was carried out and samples from the muscles, gills, eyes, liver, spleen, kidney, intestine, stomach, spinal cord and brain were fixed in 10% neutral buffered formalin. Trimmed specimens were dehydrated in rising concentrations of Ethyl alcohol, cleared in Xylol and embedded in paraffin wax. Five micron thick sections were stained by Hematoxylin and Eosin (H&E) for microscopic examination (**Bancroft and Stevens,1997**).

RESULT

1- Clinical examination of grouper fish

The collected fish samples belonged to the following grouper species:-

- Epinephelus aneus - Epinephelus guaza
- Epinephelus alexandrines - Epinephelus caninus
- Mycteroperca rubra

Despite their natural habitat on the sea bottom, most collected grouper fish were floating or swimming close to the water surface. They were swimming on one side or inverted with an opened operculum, distended abdomen and anal prolapse. In many instances, they showed

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



vertical, spinning or sleepy movement and were easy to catch. Many groupers had exophthalmia with unilateral or bilateral corneal opacity and a partial or total blindness. Their skin showed patches of pigmentation or discoloration with hemorrhagic spots and ulcers. Gills were swollen and congested. There was yellow parasitic cyst in the gills with thick mucous (Fig. 1,2,3,4,5,6).

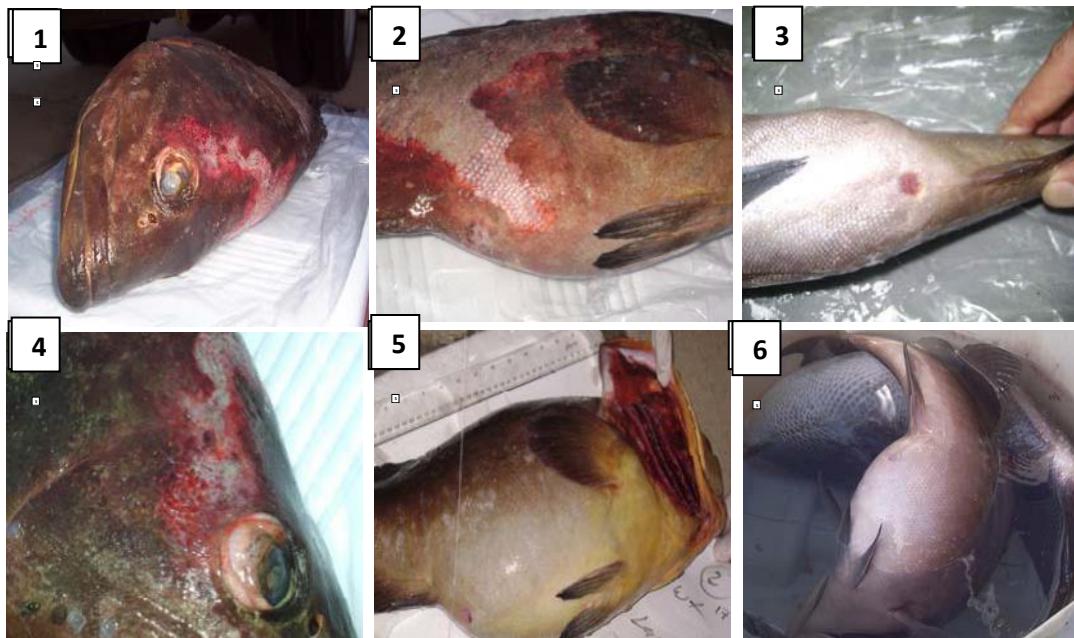


Fig. (1): Hemorrhagic patches on the head region with corneal opacity

Fig. (2): Abdominal dropsy , anal protrusion and skin hemorrhage and pigmentation .

Fig . (.3): Abdominal distension and anal inflammation.

Fig . (4): hemorrhagic spots on the skin and exophthalmia.

Fig. (5) : Severe distension of abdomen due to over inflation of swim bladder..

Fig. (6): Abnormal floating positions in affected fish.

2- Postmortem examination

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Most collected grouper fish had empty stomach and intestine .The visceral organs were swollen and hyperemic with ulcerative lesions in some cases. Mild to heavy parasitic infestation was found on the digestive tract and in the abdominal cavity and appeared as black or metallic colored cysts. In some samples, there were white nodules on the spleen, kidney and liver. The latter however, was pale in some cases. The swim bladder showed adhesions with the surrounding tissues. The abdominal cavity in some conditions contained yellowish blood-tinged turbid fluid. Fig. (7,8,9,10).

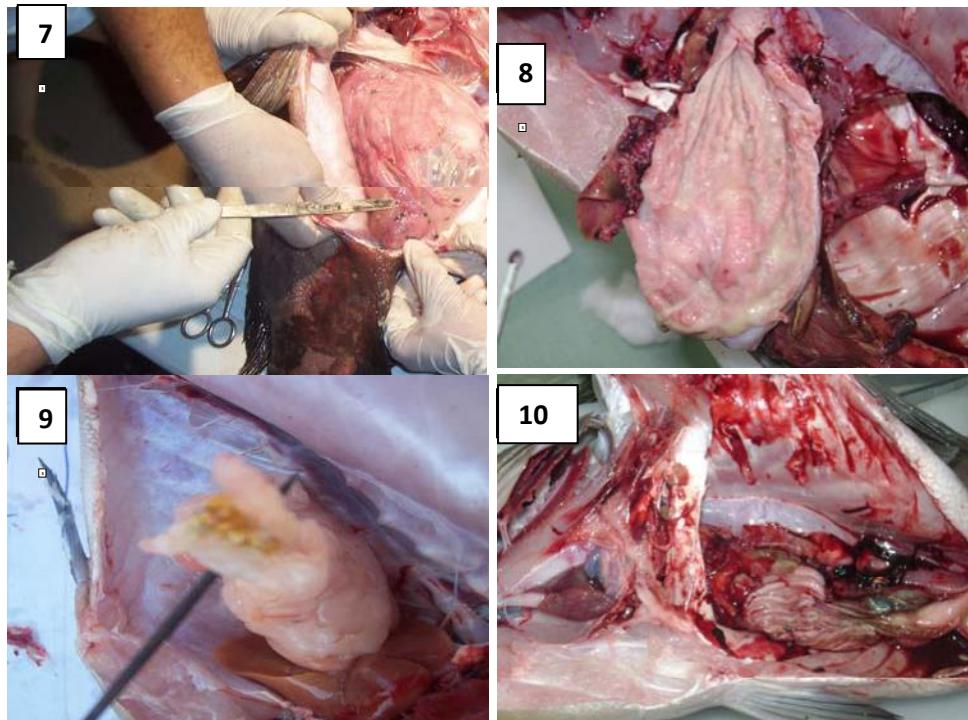


Fig.(7): Heavy infestation of abdominal cavity with parasitic cysts

Fig. (8):Empty stomach in one of the affected fish.

Fig.(9): Pus- like material with yellowish white nodules in the internal organs.

Fig. (10): Severe hemorrhage affecting the internal organs.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



3- Histopathological examination

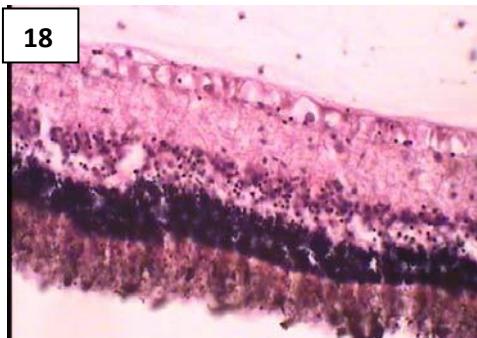
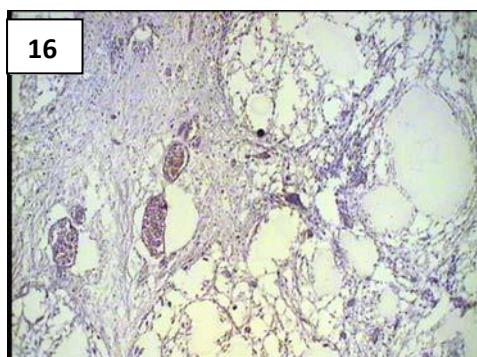
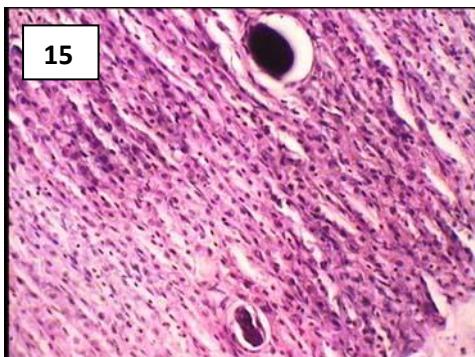
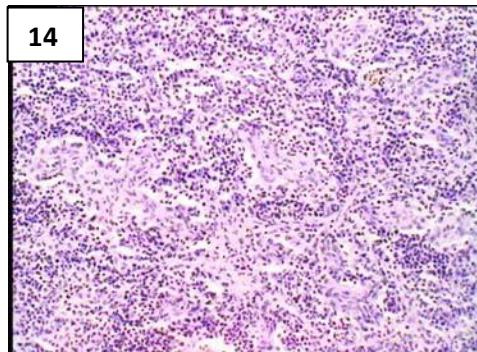
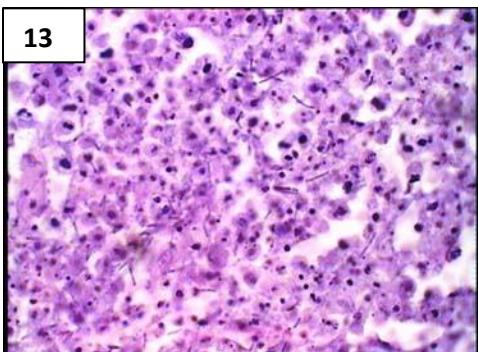
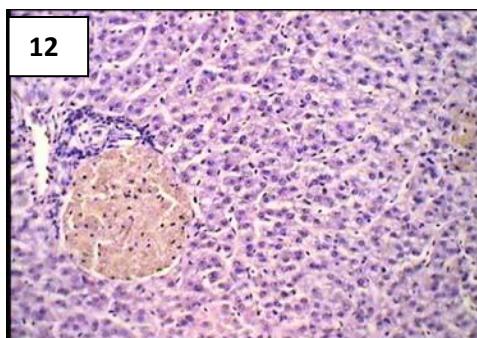
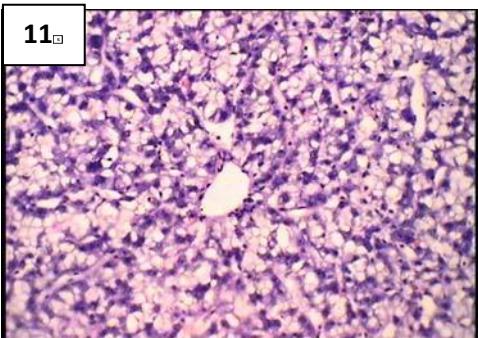
The following microscopic tissue changes existed in most of the affected fish. The liver showed fatty change(Fig.11), portal round cells aggregation, activated melano-macrophages (Fig.12) and hyaline degeneration in the vascular walls. Focal areas of necrosis with reactive leukocytic infiltration were also seen (Fig.13). Other cases revealed focal interstitial aggregations of heterophils and lymphocytes. The spleen showed depletion of the hemopoietic elements (Fig.14). Congestion, activated melano-macrophage centers and focal hematopiosis. The intestine showed increased number of goblet cells, hyperemia of mucosal blood vessels, lymphocytic infiltration in the mucosa and submucosa. Parasitic cysts were seen in the muscular coat and in the subserosa. Mature parasites and parasitic cysts were also seen in the peritoneal cavity. The stomach showed thick fibro-muscular coat, perivascular infiltration of round cells and eosinophilic granular cells. Parasitic elements were seen in the mucosa (Fig.15) and muscular layer. The brain was hyperemic and contained multiple vacuolation (malacia) (Fig.16), neuronal degeneration, neuronal vacuolation and necrosis, microgliosis and edema in Virchaw Robin space. The eyes were hyperemic with hemorrhages and telangiectasis in sclera and retina.Ulcers, calcification and hemorrhages were observed in the cornea (Fig.17). Calcification and degeneration were also seen in the inner retinal layer (Fig.18). The gills were also hyperemic with adhesion of the secondary filaments due to epithelial proliferation. It also showed round cell infiltration, focal sloughing and necrosis of gill filament epithelium. Large parasitic cysts were seen adherent to the gill filaments (Fig. 19, 20). Parasitic pancreatitis and peritonitis were seen in some cases. Hyperemia and hemorrhages of the heart along with lymphocytic myocarditis and epicarditis were most prominent changes in the heart of some cases. Parasitic cysts and melanosis were also seen in the heart. The kidneys have shown lymphocytic interstitial nephritis.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

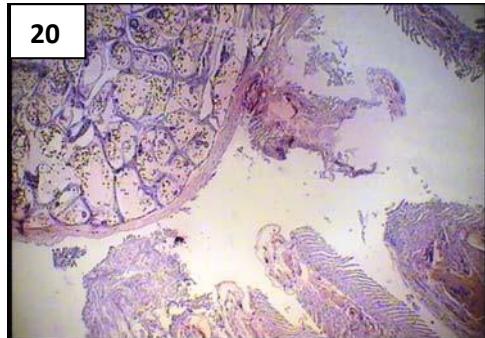
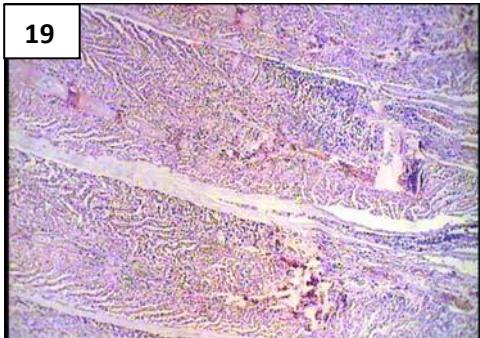


Fig.(11): Liver: Fatty change of hepatocytes (H&E x100).

Fig.(12): Liver: Round cells aggregation and activated melanomacrophage centers (H&E x100).

Fig.(13): Liver: Coagulative necrosis in hepatic tissue (H&E x100).

Fig.(14): Spleen : Focal depletion of hemopoietic elements (H&E x100).

Fig.(15): Stomach: Parasitic cysts in the mucosa (H&E x100).

Fig.(16): Brain :Congestion and multiple areas of vacuolation and malacia (H&E x100).

Fig (17):Cornea: Leucocytic infiltration ,hemorrhage and ulcer(H&E x100).

Fig.(18): Retina: Vacuolar and hydropic degeneration in the inner retinal layer(H&E x100).

Fig.(19):Gills : Leucocytic infiltration and adhesion of primary and secondary filament.(H&E x100).

Fig.(20): large parasitic cyst adherent to the gill filaments (H&E x40)

DISCUSSION

Many noxious agents have been implicated in causing disease in fish. These comprise wide range of microorganisms including *Vibrio* spp. (**Balebona et al. 1998, Ping et al. 2004**), *Streptococcus* spp. (**Evans et al. 2000**), *Micrococcaceae* (**Varvarigos,2001**) and *Photobacterium* spp. (**Mladineo et al. 2006**). A wide range of parasites has also been reported as frequent cause of disease. These include protozoans, myxozoans, microsporans, monogeneans, trematodes, crustaceans, nematodes, cestodes, acanthocephalans and hirudineans (**Nagasawa, 2004**). The related literature described different signs and symptom of infection but however, there was no mention of frank periodic nervous manifestation in the form of epidemics with serious losses due

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



to high morbidity and mortality. The current phenomenon in grouper, in particular, was recorded officially in 1985 but local fisheries have visualized such outbreaks in the region almost annually at the same period of the year. Although different types of bacteria and parasites have been recognized in the collected fish samples, the histopathologic examination revealed exciting pathological changes in the brain and ocular retina suggestive of viral infection. The nature of these histopathologic changes and their probable etiologic cause were further supported by an independent scientific institution (**Institute of Aquaculture, University of Sterling, 2008**). Confirmation of viral existence by particle isolation, direct and indirect detection or PCR were unfortunately not possible. In this study, the recorded epidemic nature, the clinical signs and the pathological picture correlates well with many reports concerning viral nervous necrosis (VNN) (**Nguyen et al. 1997, Munday et al, 2002, Yanong, 2010, and Choon-Sup et al. 2012**). This disease has a worldwide distribution. In Libya, the east coast region which harbored this phenomenon is known to be free of pollution but climatic changes have to be taken into consideration as stress factors. However, until confirming an etiological factor for this tragedy, investigation of related circumstances should be postponed. Finally our study aimed to draw the attention of specialists to consider viruses as a participating etiological factor in this phenomenon.

REFERENCES

- Arimoto, M., J. Sato, K. Maruyama, G. Mimura and I. Furusawa. 1996.** Effect of chemical and physical treatment on the inactivation of stripe jack nervous necrosis virus (SJNNV) *Aquaculture* 143:15-22.
- Balebona, M. C., Andreu, M. J., Bordas, M. A.; Zorrilla, M. I.; Morinigo, Borrego. J. J (1998):** Pathogenicity of *Vibrio alginolyticus* for Cultured Gilt-Head Sea Bream (*Sparus auratus* L.) *Appl. Environ. Microbiol.*, 65:4269–4275.
- Bancroft J.D. and Stevens A. (1977):** Theory and Practice of Histological Techniques (Book) Churchill Livingstone (Edinburgh and New York and New York)

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Bovo, G., and Florio. 2008. Chapter 4. Viral diseases of cultured marine fish. In Fish Diseases, Vol.1, Eiras, J.C., H. Segner, T. Wahli, and B.G. Kapoor, Science Publishers, Enfield, NH. pp. 202-216.

Choon- Sup, K., K. Wi-Sik, Nishizawa, Toyohiko, and Myung-joo,O.(2012): Prevalence of viral nervous necrosis (VNN) in sevenband grouper Epinephealus septemfasciatus farms. Journal of Fish Pathology 25:(2) 111-116.

Erlinda R. Cruz-Lacierda and Gregoria E. Erazo-Pagador (1996): Diseases of Cultured Grouper .AAHRI Newsletter Article from Volume 5 No.2.

Evans,J.J.; Shoemaker, C. A. & Klesius, P.H. (2000): Exeperimental Streptococcus iniae infection of hyrpid striped bass (Moron chroysops, Morone saxitilis) and Tilapia (Orechromis niloticus) by nares inoculation. Aquac.,189 :197-243.

Gomez, D.K., K. Mori, Y. Okinaka, T. Nakai, and S.C. Park. (2010): Trash fish can be a source of betanodaviruses for cultured marine fish. Aquaculture 302: 158-163.

Hartono P., Kurniastuty, Julinasari D. and Tusihadi T.(2000): Fish health and EnvironmentLaboratory National Sea farming Development center. Microbiology 146, 21-30

Hata, N., Y. Okinaka, T. Sakamoto, T. Iwamoto, and T. Nakai. (2007): Upper limits for the multiplication of betanodaviruses. Fish Pathology 42(4): 225-228.

ICTV (International Committee on Taxonomy of Viruses) online, Nov. 24, 2010.

Institute of Aquaculture " Veterinary Diagnostic Center" University of Sterling, FK9 4LA, Scotland, UK. Nov. 2008. (Report).

Kai,Y., H. Su, J. Tai and Chi. 2010. Vaccination of grouper broodfish (Epinephelus tukula) reduces the risk of vertical transmission by nervous necrosis virus. Vaccination 28: 996-1001.

Leong, T.S. (1994): Parasites and Diseases of Cultured Marine Fin fishes in South East Asia.Percetakan Guan, Malaysia.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Mladineo I, Miletic I, Bocina I (2006) : Photobacterium damsela subsp Piscicida outbreak in cage-reared Atlantic bluefin tuna Thunnus thynnus. J Aquat. Anim Health 18:51–54

Munday, B.L., J.Kwang, and N. Moody. (2002). Betanodavirus infections of teleost fish: a review. Journal of Fish Diseases 25:127- 142.

Nagasawa K. (2004): The Regional Fish Disease Project Southeast Asian Fisheries Development Center, Aquaculture Department Tigbauan 5021, Iloilo, Philippines, December.

Nagasawa K. and Erlinda R. Cruz-Lacierda(2004):Southeast Asian Fisheries Development Center, Japan Aquaculture Department.

Nguyen, H.D., K. Mushiake, T. Nakai, and K. Muroga. (1977). Tissue distribution of striped jack nervous necrosis virus (SJNNV) in adult striped jack. Diseases of Aquatic Organisms 28:87-91.

Ping, C. L.; Lin, J. Y.; Hsiao, P.T. & Lee, K. K. (2004): Isolation and characterization of pathogenic Vibrio alginolyticus from diseased cobia Rachycentron canadum L. J. Basic Microbiol., 44: 23–28.

Varvarigos, P. (2001): Gram positive cocci-bacteria, Micrococcaceae, Streptococcaceae, causing systemic disease in intensively farmed fish. December.Varvarigose .Athens, Greece.

Yanong, R.P.D(2010).: Viral Nervous Necrosis (Betanodavirus) Infection in Fish. Program in Fisheries and Aquatic Sciences, University of Florida. (FA180).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



BILATERAL CERUMINOUS GLAND ADENOCARCINOMA IN A CAT

Mahir AG Kubba¹, Saeed N. Wafa ²and Seham A. Al-Azreg ¹

1- Department of Pathology, Faculty of Veterinary Medicine, University of Tripoli.

2- Department of Surgery, Faculty of Veterinary Medicine, University of Tripoli.

ABSTRACT

Recurrent bilateral ceruminous gland adenocarcinoma was diagnosed in a nine years old Persian-mix female cat. The tumor nodules were first noticed in the external ear canal 5 years ago and were resected surgically 2 years after their discovery. Reoccurrence was further reported on two occasions, i.e at 7 months and 3 months respectively before the publication of this article and were surgically resected as well. The tumor cellular morphology was described and discussed. This article documents ceruminous gland adenocarcinoma in cat for the first time in Libya.

Key words: Feline tumors, Feline ear tumors, Ceruminous gland adenocarcinoma.

INTRODUCTION

Ceruminous glands are modified apocrine tubular sweat glands located in the external auditory canal. Its secretion combines with that of the sebaceous glands to form cerumen which is a brown waxy material that protect the ear canal and keeps the tympanic membrane moist and pliable (**Banks, 1981**). Abnormalities in the ceruminous glands are infrequent but their impact ranges from mere discomfort to life threatening. Many lesions have been reported including ceruminous gland cysts in cats as well as glandular hyperplasia, dysplasia, adenoma and adenocarcinoma in dogs and cats (**Withrow and Vail, 2007**). Among other types of tumors in the ear canal, ceruminous gland adenoma and adenocarcinoma are most commonly encountered in dogs and cats where malignancy predominates in the later (**Moisan and Watson, 1996**). The usual development of these tumors secondary to otitis externa may suggest a role for inflammatory process in their initiation. Otitis externa and interna, on the other hand, may be

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



established following auditory canal tumors which are completely obstructive (**London et al. 1996**). Others (**Merck, 2011 and Gotthelf, 2005**) have suggested that inspissated apocrine secretions from hyperplastic ceruminous glands may stimulate carcinogenesis in the ear canal. Ceruminous gland adenocarcinoma is locally invasive and may metastasize to regional lymph nodes and to the parotid salivary gland. Distant metastasis to the lung is rare (**Merck Sharp and Dohme, 2011**)

The current paper is intended to investigate the pathology of a bilateral ceruminous gland adenocarcinoma in a cat which showed reoccurrence after two surgical excisions.

MATERIAL AND METHODS

Bean sized nodules were removed surgically under general anesthesia. They were examined grossly and preserved in 10% neutral buffered formalin (NBF) for 48 hours. Specimens were then trimmed, dehydrated in increasing concentration of Ethyl alcohol, cleared in Xylol and embedded in Paraffin wax. Sections of 5-6 microns were stained by Hematoxyline and Eosin (H&E) for microscopic examination (**Lillie, 1965**).

Case history

A nine years old Persian-mix female cat was presented with bilateral ear complaint, otorrhea, head shaking, rubbing and scratching of ear regions associated with inappetence and depression. She had bilateral exudative otitis externa along with multiple bean-sized dome-shaped nodules which have nearly blocked the outer auditory canal in both sides. The nodules were removed surgically but reoccurrence took place four months latter. This time, the growth appeared as a single nodule on the left ear canal (Fig.1).This cat has had similar nodules five years ago and were removed surgically two years after their discovery.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



RESULTS

The nodules in both occasions were hard, ulcerated and had grayish-white cross section. Microscopic examination revealed a non capsulated subcutaneous dense area of glandular and mesenchymal elements. The glandular element showed different pathologic changes which ranged from hyperplasia and metaplasia to dysplasia and neoplasia. Most acini were voluminous with irregular configuration and displayed back to back arrangement, and though separated by layers of hyperplastic fibroblast and myoepithelium, protrusion into the surrounding stroma was frequent (Fig.2). These acini displayed different epithelial cell orientations. They were fully packed with large polygonal epithelial cells which occasionally contained central area of necrosis (Fig. 3) or displayed cribriform cellular arrangement due to the existence of secondary intraepithelial acini (Fig. 4). In many instances, the glandular epithelial cells were vacuolated and contained golden brown granules of cerumen, large nuclei and prominent nucleoli with occasional mitotic figures (Fig.5). Glands with less extensive changes were also seen. Likewise, they were irregular but were lined by atypical columnar epithelium of frequent folding, nuclear stratification and occasional squamous metaplasia (Fig. 6). Solid masses of poorly differentiated cells invading the stroma were also noticed especially at the edges of the tumor mass. These cells were highly pleomorphic, hyperchromatic and contained plenty mitotic figures (Fig.7). The granulation tissue which replaced the damaged skin in many locations was heavily infiltrated with neutrophils of secondary bacterial infection. Neutrophils have also imposed remarkable damage on neoplastic cells which are located close to the surface. By comparison, the recently excised nodule showed an overwhelming domination by poorly differentiated glandular cells which have arranged into solid infiltrating clusters.

DISCUSSION

The true incidence of ear canal tumors in cats is not known, but based on surveys of total submissions to pathology laboratories, less than 2% of all tumors in cats occur in the ear canal.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



The most common tumors, however, are ceruminous gland adenoma and adenocarcinoma. Other tumors include squamous cell carcinoma, mast cell tumor, malignant melanoma, hemangiosarcoma, fibrosarcoma lymphoma and basal cell carcinoma (**Moisan and Watson, 1966, Rogers, 1988**). Identification of ceruminous gland adenoma and adenocarcinoma is basically dependant on the location and the gross and microscopic appearance. Immunohistochemical localization of specific tissue markers is employed especially in man for further confirmation. The existence of wide range of cellular changes which varied from hyperplasia and dysplasia to benign and malignant transformation agreed well with the finding of others (**Withrow and Vail, 1966, Merk Sharp and Dohme, 2011**). An explanation for the diversity in cellular pathological change was basically dependant on a preceding chronic otitis media (**Gotthelf, 2005**). In the later, the associated increase in lipofuscin-laden phagocytes provide continued cytokine and growth factor production which may contribute to perpetuation of glandular hyperplasia. Hyperplasia along with chronic inflammation may predispose for cellular transformation into adenoma and adenocarcinoma. In our case, there was profuse intracellular and extracellular cerumen along with lipofuscin-laden macrophages. The progression in malignancy noticed in the last biopsy is consistent with the basic knowledge about recurrent neoplasms (**Moulton, 2002**).

It has been stated that conservative ear resection of ceruminous gland adenocarcinoma is expected to provide a 10 months disease- free interval, a 60% recurrence rate and a 33% 1 year survival (**Mariano, et al. 1994**). Our cat has first had her nodules 5 years ago and was undergone conservative resection on three occasions that is 2 years after its discovery, 7 months and 3 months respectively before preparing this manuscript. The relative long time of survival probably indicate that these nodules may have originated due to glandular hyperplasia and/or ectasia which may take variable durations to transform into adenocarcinoma (**Gotthelf, 2005**).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



REFERENCES:

Banks, WJ 1981: Applied Veterinary Histology. (Book) Williams and Wilkins, Baltimore London. 1st. ed.: P.533,

Gotthelf, LN 2005: Small animal ear diseases: An illustrated guide. 2nd edition, Elsevier 64-73.

Lillie R.D 1965: Histopathologic technique and practical histochemistry. 3rd edition Blakiston Division, McGraw-Hill.

London CA, Dubilzeig RR, Vail DM et al 1996: Evaluation of dogs and cats et al: Evaluation of dogs and cats with tumors of the ear canal: 145 cases (1978-1992), J Am Vet Med. Assoc 208:1413-1418.

Mariano DJ, MacDonald JM, Matthiesen DT et.al 1994: Results of surgery in cats with ceruminous gland adenocarcinoma, J Am Anim. Hosp. Asso. 30: 54-58,

Merck Sharp and Dohme Corp. 2011: Tumors of the skin and soft tissues: a subsidiary of Merck & Co. Inc. Whitehouse Station, NJ.USA.

Moulton JE (2002): Tumors in domestic animals. 4th ed. St. University press , Ames, Iowa.

Moisan PG, and Watson GL 1996: Ceruminous gland tumors in dogs and cats: a review of 124 cases. J Am. Anim. Hosp. Assoc. 32: 449-453.

Rogers KS 1988: Tumors of the ear canal, Vet. Clin. North Am. Sm. Anim. Pract. 18(4): 859- 868.

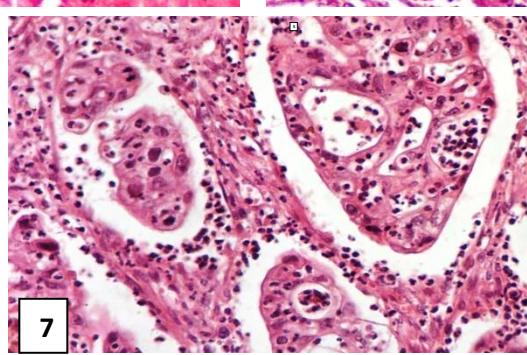
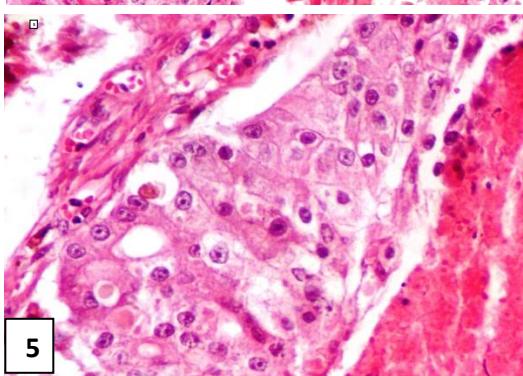
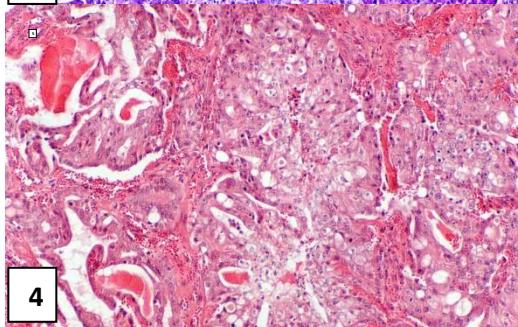
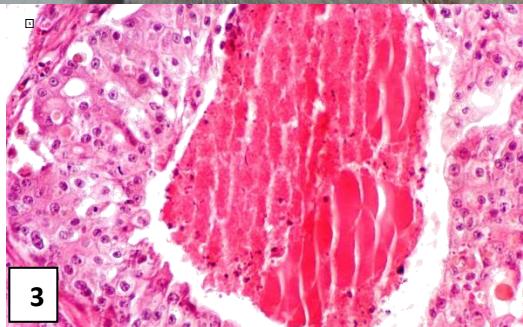
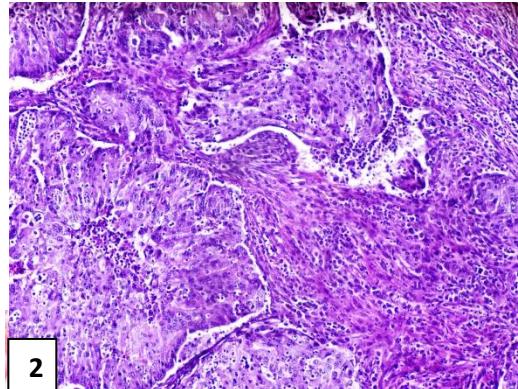
Withrow SJ and Vail DM 2007: Withrow and MacEwen's Small animal clinical oncology (book), Saunders Elsevier, 4th ed. 393-394.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Figure Legend

Fig.1: A chick pea- sized ulcerated tumor mass almost occluding the entrance of the left ear canal.

Fig.2: Irregular voluminous cell packed acini separated by intense myoepithelial proliferation (H&E, x10).

Fig.3: A large glandular acinus packed with large polygonal epithelial cells and shows a central area of necrosis (H&E, x20)

Fig.4: An acinus showing cribriform cellular arrangement due to the existence of secondary intraepithelial acini (H&E, x10).

Fig.5: Vacuolated glandular epithelial cells containing golden brown granules of cerumen (arrow), large nuclei and prominent nucleoli(H&E, x40).

Fig.6: Glandular acini are lined by atypical folded columnar epithelium, nuclear stratification and squamous metaplasia(H&E, x20).

Fig.7: Solid masses of poorly differentiated cells invading the connective tissue. These cells are highly pleomorphic, hyperchromatic with plenty of mitotic figures. (H&E, x20).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Patho-physiological Studies On the Effects of Confidor (Imidacloprid) in Albino Rats

¹Mouchira M. Mohi El-Din, ²Abd-EL-Raham A. El-shater and ²Rana A. Ali Ahmed

¹Pathology and Clinical Pathology Department, Faculty of Veterinary Medicine,

²Zoo Department, Faculty of Science, South Valley University, Qena.

ABSTRACT

Thirty two adult female rats, at aged 2- 3 months and weighted 150- 200g, were divided into four groups (n= 8 rat). Groups 1, 2 and 3 were orally received (45 mg/ kg of body weight (b.wt)) of confidor at a dose 1/10 of LD₅₀ every two days for 3, 6 and 9 times, respectively. The group (4) was served as control. All administrated rats were sacrificed after the last dose of treatment i.e. days (6, 12 and 18 of treatment), respectively. Blood samples were collected for hematological and biochemical parameters. Specimens from liver, kidneys and spleen were collected for histopathological examination. The hematological results revealed a significant decrease in total RBCs count, Hb conc and HCT percentage after high doses of confidor. The biochemical findings showed a significant increase in glucose, total proteins, uric acid and creatinine, while cholesterol was significantly decreased in high doses of confidor. The plasma AST, ALT activities showed significant decreased in the rats which received confidor with different doses. Histopathology, showed degenerative changes and necrosis in the liver and kidneys, while the spleen displayed depletion in lymphoid cells with hemorrhage in different doses of the insecticide. It could be concluded that confidor has toxic effects on the visceral organs at the different doses, besides impaired liver and kidneys functions.

Keywords: Albino rats; Confidor; Pathology; Physiology; Toxic effect.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



INTRODUCTION

The main purposes of utilization of pesticides are in agriculture, public health and personal use. Confidor (imidacloprid) is a nitroguanidine synthesized compound, produced by Nihon Bayer in Japan (**Leicht, 1996**). The commercial name of this type is Admire, Gaucho, Premise, Provado and Marathon, all contains imidacloprid as the active ingredient (**Sayed, 2001**). Confidor belongs to a new chemical class of insecticides: the chloronicotinyls, syn-neonicotinoids. Confidor is considered to be a member in the neonicotinoid class of insecticides which are representatives of the chloronicotinyl (**Yamamoto et al., 1995**). **Parwinder et al., 2001** reported that the new insecticide confidor become available for use against white grubs in turfgrass. Neonicotinoid appeared to interfere with the nicotinic acetylcholine receptors located in the postsynaptic membrane of insects (**Bai et al., 1991**). In the past, knew that confidor induced destruction in the renal tissues with hyperaemic blood vessels but recently, noticed that confidor was introduced as a pest control agent against sucking insects in Egypt (**El-Tarra et al., 1995**). The present work aimed to investigate the hematological, biochemical and histopathological effects of confidor on albino rats after oral administration.

MATERIALS AND METHODS

Chemicals

Confidor is a chloronicotinyl insecticide. The common name is imidacloprid and the chemical name is (1- {((6-Chloro-O-Pyridinyl) methyl}-N-nitro-2-imidazolidinimine). It was produced by Bayer AG chemical company. The formula used in the present study was confidor 200 SC.

Experimental animals

Thirty two adult female albino rats (aged 3 months and weighted about 150- 200 g) were used in this study. They were maintained in cages (8 rats/ cage) in an air-conditioned room, they were fed on adequate stable commercial balanced diet and examined daily for two weeks, prior to beginning of experiment for free from infection.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Experimental design

The experimental animals were randomly divided into four groups ($n= 8$). The groups 1, 2 & 3 were orally received confidor at dose (45 mg/ kg of body weight, at a dose 1/10 of LD50) day by day 3, 6 & 9 times respectively. The group 4 was served as control and did not subject to any treatment. At the last dose of the insecticides; the animals which were treated with 3 doses of the insecticides were sacrificed at the 6th day, while the animals which were treated with 6 & 9 doses, of the insecticide were sacrificed at the 12th and 18th days from the beginning of the experiment, respectively. The blood was collected in clean EDTA tube for hematological analysis. Plasma was separated and used for biochemical analysis.

Hematological analysis

Blood profiles including Red blood cell count (RBCs), differential leukocytic count (WBCs), Hemoglobin concentration (Hb%), and hematocrit ratio (HCT) were measured using automatic hematology analyser. All samples were measured by COBAS micro-apparatus. The apparatus provided total RBCs, WBCs, Hb and HCT for each sample and provided the mean value. The apparatus was calibrated at the beginning of each determination according to manufacturer instructions using controls of known values.

Biochemical analysis

- 1- Plasma glucose was determined by using a spectrophotometer randox kits (United Kingdom) according to **Trinder, 1969**.
- 2- Plasma cholesterol was determined by enzymatic method as described by **Richmond, 1973**.
- 3- Plasma triglycerides were determined by enzymatic calorimetric method as described by **Young et al., 1975**.
- 4- Plasma total proteins were performed by the Biuret method as described by **King and Wootton, 1964**.
- 5- Albumin and globulin were determined by calorimetric method using bromocresol by **Doumas et al., 1971**.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



6- Plasma uric acid was determined according to the method described by **Barham and Trinder, 1972**.

7- Plasma creatinine was determined by kinetic method as described by **Hare, 1950**.

8- Plasma alanine Transaminase (ALT) and aspartate transaminase (AST) were determined by colorimetric test according to **Bergmeyer et al., 1978**.

Histopathological examination

Clinical signs and post-mortem changes were recorded. Specimens from liver and kidneys and spleen were collected from the sacrificed animals. The tissue sections were kept in 10% neutral buffered formalin and processed at 5 μ thick paraffin. The tissues were stained with hematoxylin and eosin for histopathological examination.

Statistical Analysis

The data was statistically analyzed by one-way analysis of variance (pc-state computer program) and the least significant difference (L.S.D) was used to test the difference between treatments. Results were considered statistically significant when $P < 0.05$

RESULTS

Blood hematological examination

The results recorded in Table 1 revealed that total RBCs, WBCs and Hb concentration in female albino rats treated orally day by day with 45 mg/kg b.wt of confidor for 6 days were insignificantly changed. There was highly significant increase ($P < 0.01$) in HCT percentage. The rats which received the insecticide for 12 days recorded highly significant decrease in Hb conc insignificant changes in RBCs, WBCs and HCT. Whereas, after 18 days receiving the insecticide and insignificant changes in WBCs and highly significant decrease in RBCs counts, HCT percentage and Hb concentration ($P < 0.01$) were recorded when compared with the control group.

Plasma biochemical analysis

As shown in Table 2 the concentration of plasma glucose in female albino rats treated orally day by day with 45 mg/kg b.wt of confidor for 6, 12 and 18 days was highly significantly increased ($P < 0.01$). Cholesterol levels showed insignificant changes in the rats which received the confidor

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



for 6 days, while after 12 and 18 days of treatment, cholesterol was highly significantly decreased but triglyceride levels were highly significantly increased ($P<0.01$). In Table 3 no statistically significant change was observed in the total proteins and their constituents (albumin and globulin) in the rats which received the confidor for 6 days, while after 12 days of treatment, they were highly significantly increased. After 18 days of treatment, the plasma total proteins and globulin revealed insignificant change and the albumin increased in a significant manner. Moreover, uric acid and creatinine showed highly significant increase ($P<0.01$) in the rats which received the confidor for 6, 12 and 18 days. In other hand, Table 4 showed a highly significant decrease ($P<0.01$) in plasma AST and ALT levels in the rats which were received the confidor for 6, 12 and 18 days when compared with the control.

Pathological findings

Clinical signs and gross appearance

Clinically, no remarkable signs of toxicity were showed in most rats received the confidor for 6, 12 and 18 days. Grossly, congestion with enlargement was noticed in the liver and spleen with dark red spots on the surface of kidneys in most rats which received confidor mainly for 18 days.

Histopathology

The hepatic lesions were severe and involved the majority of the hepatic parenchyma. The liver showed aggregation of mononuclears mainly lymphocytes, besides fibroblast cells proliferated around portal areas and the blood vessels were engorged with blood and some mononuclears mainly neutrophils and lymphocytes were observed inside the lumen (**Fig. 1**). Proliferation of fibroblast cells was extended to interlobular tissues particularly in rats that received the confidor substance with (1/10 LD50) 6& 9 times for 12 and 18 days. Moreover, telangiectasis characterized by dilation and engorgement of hepatic sinusoids with blood was noticed and induced pressure atrophy in the neighboring hepatic cells which were replaced with leukocytic cells mainly lymphocytes, macrophages and fibroblast cells with hemosiderosis among the hepatic cells (**Fig. 2**). Cholangitis characterized by aggreagation of mononuclears and fibroblasts around the bile ducts was mainly seen in the rats which received the confidor 6 and 9 times for 12 and 18 days

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



(**Fig. 3**). The kidneys displayed degenerative changes in the renal tubules in most rats which received the confidor with different days. The kidneys showed necrosis of the renal tubules with hemorrhage, besides edema of the Bowman's capsule leading to shrinkage of the glomerular tufts and dilated of Bowman's space (**Fig. 4**). These lesions were showed in the rats which received 9 doses of confidor (1/10 LD50) for 18 days. In addition to, the capsule was thickened with fibrous connective tissue proliferations (**Fig. 5**). Renal casts were displayed in the collecting renal tubules mainly in the rats which received 9 doses of the insecticide for 18 days (**Fig. 6**). Endotheliosis in the endothelial cells lining the renal blood vessels with thickening and hyalinization in the smooth muscles and perivascular edema were evident in some rats that received different doses of confidor. Acute ureteritis was manifested with leukocytic cells mainly lymphocytes and macrophages was noted in the subepithelial tissues and accumulated in the adventitia, besides thickening and hyalinization in the smooth muscle fibers of the muscular layer (**Fig. 7**). Depletion and necrosis of the lymphocytes in the white pulp of spleen with hemorrhage among the splenic tissues and subcapsular were common in the rats received the substance with different doses, besides thickening and hyalinization in the splenic blood sinusoids (**Fig. 8**).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

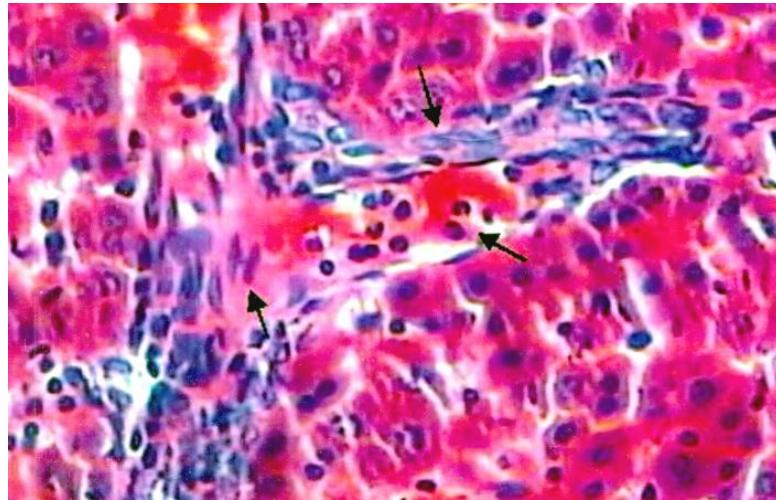


Fig. 1: Liver of the rats (6 times of confidor) for 12 days showing aggregation of mononuclears mainly lymphocytes, besides fibroblast cells proliferation around portal areas and the blood vessels were engorged with blood. (H&E.,x 80)

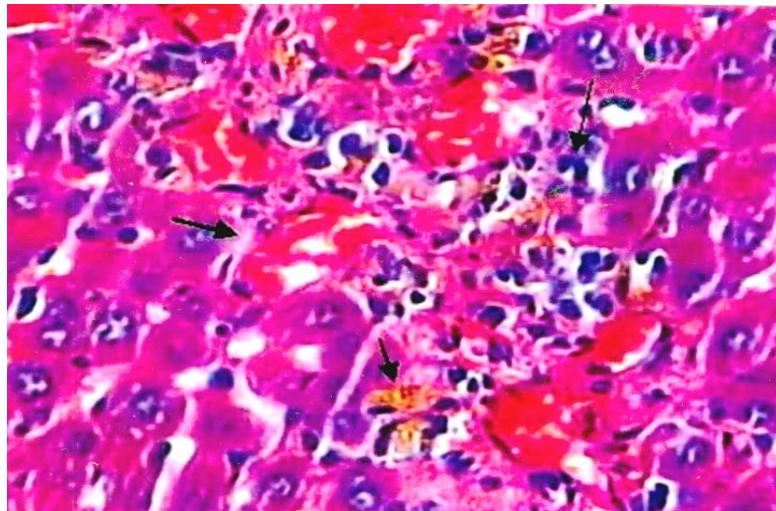


Fig. 2: Liver of the rats (9 times of confidor) for 18 days showing telangiectasis characterized by dilation and engorgement of hepatic sinusoids with blood and pressure atrophy in the neighbouring hepatic cells which were replaced by leukocytic cells. (H&E.,x 80)

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

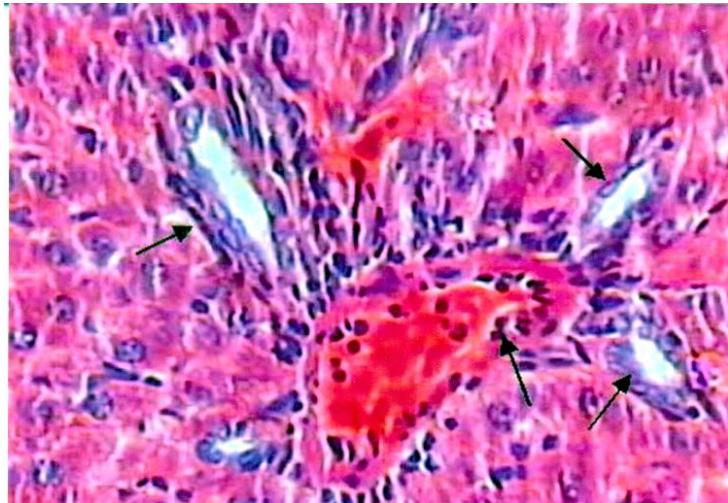


Fig. 3: Liver of the rats (9 times of confidor) for 18 days showing cholangitis characterized by aggregation of mononuclears with fibroblasts around the bile ducts. (H&E, x 80)

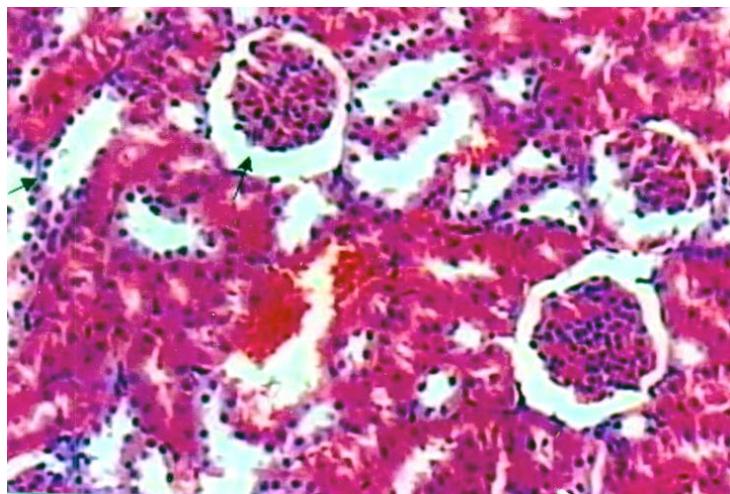


Fig. 4: Kidneys of the rats (6 times of confidor) for 12 days showing shrinkage of the glomerular tufts and dilated Bowman's space, besides necrosis in most of renal tubules. (H&E, x 40)

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

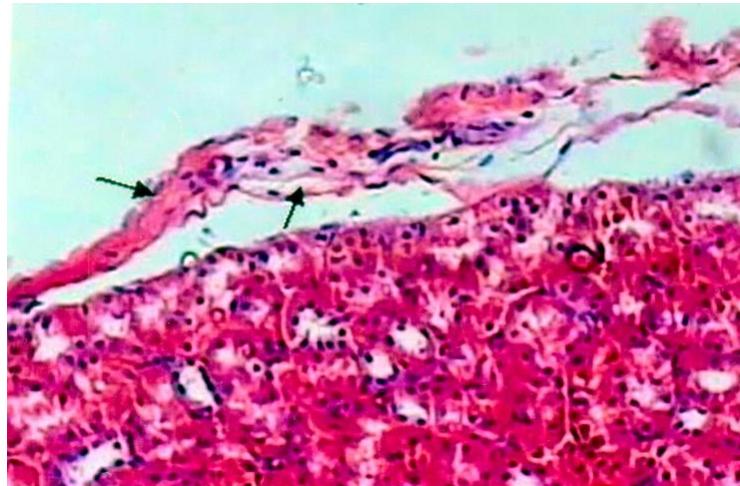


Fig. 5: Kidney of the rats (9 times of confidor) for 18 days showing thickening in the renal capsule with fibrous connective tissue proliferations. (H&E.,x 40)

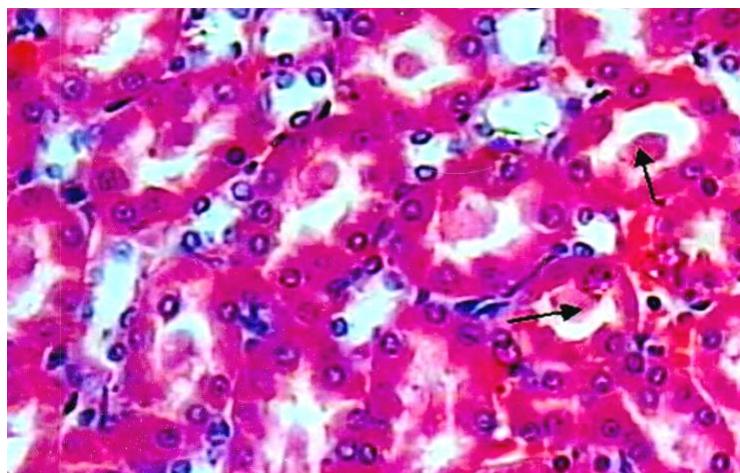


Fig. 6: Kidney of the rats (9 times of confidor) for 18 days showing renal casts displayed in the collecting renal tubules. (H&E.,x 80)

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

**Hematological findings:****Table (1):** Effect of oral administration of different doses of insecticide (confidor) on some haematological parameters of female rats.

Groups Parameters	Control group	3 doses confidor	6 doses confidor	9 doses confidor
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
WBCs x (10 ³ /mm ³)	10.08±0.51	9.81±0.37	10.02±0.31	10.31±0.67
RBCs x(10 ⁶ /mm ³)	6.74±0.40	6.91±0.52	6.34±0.20	5.92±0.22--
HCT (%)	34.9±1.14	37.1±0.44	35.26±0.85	33.47±0.56--
Hb (gm/dl)	12.31±0.60	11.85±0.19	11.46±0.32	11.12±0.20--

The results are expressed the mean ± S.D. of 8 animals,

+ Highly significant increase (P<0.01).

-- Highly significant decrease (p< 0.01).

Table (2): Effect of oral administration of different doses of insecticide (confidor) on plasma glucose and cholesterol and triglycerides levels of female rats.

Groups Parameters	Control group	3 doses confidor	6 doses confidor	9 doses confidor
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Glucose (mg/dl)	48.20±4.714	64.21±4.951 ⁺	70.00±2.09 ⁺⁺	74.54±2.405 ⁺⁺
Cholesterol (mg/dl)	87.70±8.95	84.28±6.69	68.33±5.54 ⁺	66.55±3.82 ⁺⁺
Triglycerides (mg/dl)	63.15±8.43	89.86±3.8T4 ⁺⁺	155.21±2.57 ⁺⁺	96.30±8.55 ⁺⁺

The results are expressed the mean ± S.D. of 8 animals.

+ highly significant decrease (P<0.01).

++ highly significant increase (P< 0.01).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Table (3): Effect of oral administration of different doses of confidor insecticide on plasma on plasma total proteins, albumin, globulin, uric acid and creatinine levels of female rats.

Groups Parameters	Control group	3 doses (G ₁)	6 doses (G ₂)	9 doses (G.O)
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Total proteins (mg/dl)	5.17±0.99	4.82±0.32	7.09±0.24 ⁺⁺	5.08±0.17
Albumin (ing dl)	2.65±0.24	2.51±0.15	4.18±0.13 ⁺⁺	2.84±0.14
Globulin (ing dl)	2.14±0.56	2.31±0.29	2.91±0.25	2.24±0.27
Uric acid (mg/dl)	4.13±0.12	6.03±0.37 ⁺⁺	10.34±0.31 ⁺⁺	7.03±0.69 ⁺⁺
Creatinine (mg/dl)	1.18±0.12	2.23±0.33 ⁺⁺	2.49±0.26 ⁺⁺	2.47±0.19 ⁺⁺

The results are expressed the mean ± S.D. of 8 animals.

⁺ highly significant decrease ($P < 0.01$).

⁺⁺ highly significant increase ($P < 0.01$).

Table (4): Effect of oral administration of different doses of confidor insecticide on plasma GOT and GPT levels of female rats.

Groups Parameters	Control group	3 doses (G ₁)	6 doses (G ₂)	9 doses (G ₃)
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
GOT (u/L)	179.87±13.89	100.62±12.92 ⁺⁺	147.12±10.28 ⁺⁺	123.25±10.20 ⁺⁺
GPT (u/L)	24.25±2.81	13.37±2.72 ⁺⁺	16.25±3.57 ⁺⁺	19.37±3.46 ⁺⁺

The results are expressed the mean ± S.D. of 8 animals.

⁺⁺ highly significant decrease ($P < 0.01$).

DISCUSSION

The present study revealed a significant decrease in RBCs counts as well as Hb conc when 9 doses of confidor insecticide administrated within 18 days, while HCT percentage was increased

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



after 3 doses and decreased after 9 doses of administration. The reduction of RBCs and Hb conc is similar to the results induced by methomyl and tricholorafon in pigeon and chicken (**Saleh and El-Saify, 1990**), by lannaate in rats (**El-Missiry and Othman 1993**) and dimethoate in toad (**El- Bakatry et al, 1993**). The depression in RBCs, Hb and HCT percentage declared that confidor may be anemic. This was noticed in the highly blood loss as results showed red cells destruction by haemolytic agents or rapid cell removal due to the over activity of spleen, which detected histological, in hemorrhage among splenic tissue. This decreasing may be due to a hemolytic action through the destruction of protein and mucopolysaccharide structures of the walls of the blood vessels affected by pollutants and may be due to the change diminution of RBCs from the circulation as a result of extravagation of the blood induced by pesticides (**Assem et al., 1992**). Moreover, the decrease in RBCs may be due to a reduction in the production and output of cells from hemopoitic site (**Khadre, 1988**). The decrease in HCT percentage after 9 doses may be attributed to the significant decrease in RBCs and /or shrinkage of RBCs.

Some significant changes in plasma glucose were observed in the present work compared to its values in control group. Glucose was a significantly increased. Its attributed to the depression of the liver glycogen formation and elevated concentration of serum glucose (**Rao and Rao, 1984; Gluth and Hanke, 1985**). **Gupta, (1974)** stated that the hyperglycemia of rats induced by malathion might be explained in part by inhibition of cholinesterase at the neuroeffector sites in the adrenal medulla leading to hypersecretion of adrenaline which, stimulated the breakdown of glycogen glucose. Moreover, it may be due to release of epinephrine from adrenal medulla, in response to toxin that enables the animals to mobilize their resources quickly for metabolic requirement (**Apple et al., 1995**). Others studies suggested that insecticides increased the glucose due to endogenous insulin release due to a damage of pancreatic tissue (**Eman et al., 1997 and El-Hennawy et al., 1980b**).

The effect of confidor on plasma lipid (cholesterol and triglycerides) was not changed after 3 doses. The level of cholesterol decreased after 6 and 9 doses but triglycerides was highly significantly increased in all given doses. This may be attributed to the inhibition of acetyl CoA,

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



which represents the precursor agent in the lipid fraction biosynthesis, then the cholesterol biosynthesis may be inhibited or the rate of its catabolism was stimulated by insecticide administration (**Abdel-Rahim and Eweis, 1995**). **Patyza et al., (1965)** assumed that the reduction in cholesterol might be the result of disturbance in carbohydrate metabolism. The disturbance in carbohydrate metabolism might be induced by a decrease in the lipid content due to active oxidation and utilization of lipids (**Abdel-Raheem et al., 1986**). **El-Hennawy et al., (1980a)** reported that a decrease of serum cholesterol not only due to hepatotoxic effect but also due to hyperfunction of the thyroid.

The increasing of the level of serum total protein noticed in the present study may be due to hypertrophy occurred in liver cells (**Mitjavila et al., 1981**).

The effect of confidor on plasma uric acid and creatinine levels in rats showed significant increase in all given doses. Serum uric acid and creatinine can be used as a rough index of the glomerular filtration rate (**Hernandez and Coulson, 1967**). High value of uric acid and creatinine indicate several disturbances in kidney (**Maxine and Benjamin, 1985**). The observed increase in plasma uric acid may be attributed to the action of confidor insecticides which causes pathological changes in the kidneys. Moreover, the accumulation of insecticides in the kidneys may cause malfunction and damage of the renal cells followed by an increase in serum creatinine and uric acid levels (**Marie et al., 1998**).

The transaminases enzymes (AST and ALT) activities are often considered to be important in the assessment of the state of the liver as well as of some other organs and considered as specific indicators of liver damage (**Uppal and Ahmed, 1977**). In the present study, the confidor decreased the activities of the enzymes after given all doses. It may be due to the biochemical dysfunctions and lesions of the liver tissue caused by the confidor (**El- Mahrouky et al., 2001**). It could be concluded that confidor has toxic effects on the visceral organs with the different doses, besides impaired liver and kidney functions.

REFERENCES

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Abdel-Raheem K, El-Elaimy I, El-Mossallamy N and El-Mahrouky F (1986) : Induced toxicity of anticoagulant rodenticides IV. Changes in lipid metabolism in rats. Proc. Zool. Soc. A. R. Egypt, (10): 21- 29.

Abdel-Rahim EA and Eweis EA (1995): Comparative toxicity of pure and formulated parathion in albino rats .1. growth rate, thyroid function, liver glycogen, blood lipid fraction and sugar. J. Agric. Sci. Mansoura Univ., 20(5): 2525- 2533.

Apple JK, Dikeman ME, Minton JE, McMurphy RM, Fedde MR, Leith DE and Unrah JA (1995) : Effects of restraint and isolation stress and epidural blockade on endocrine and blood metabolite status, muscle glycogen depletion, and incidence of dark-cutting longissimus muscle in sheep J. Anim. Sci. 73:2295

Assem H, Abo-hegab SL and Belal E (1992): comparison of hematological effects of some toxicants on Clarias goriepinus. J. Egypt. Ger. Soc. Zool., 9 (A), Comparative physiology, 33-50.

Bai D, Lummis SCR, Leicht W, Breer H and Sattelle DB (1991) : Actions of imidacloprid and a related nitromethylene on cholinergic receptors of an identified insect motor neurone. Pestic. Sci., 33 (2) 197–204

Barham D and Trinder P (1972): Determination of uric acid in serum enzymatic colorimetric method. Analyst, 97: 142.

Bergmeyer HU, Scheibe P and Wahlefeld AW (1978) :Optimization of methods for aspartate transaminase and alanine transaminase. Clin. Chem. 24, 58-73

Doumas BT, Watson WA and Biggs HG (1971): Albumin standards and the measurement of serum albumin with Bromcresol green. Clinica chim. Acta. 31: 87–96.

El- Bakary AS, Abdel Gawad AF, El-Mofty MM and Atia SI (1993): Effect of an organophosphate insecticide, dimethoate on some hematological parameters of toads *Bufo regularis*. 4th annual meeting od Saudi Biol. Soci. King abdulaziz Univ., Jeddah, (Abstract) p., 111

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



El-Hennawy SI, Shama AI, Geabah NE and Sadek M (1980a): The effect of some herbicidal agents on biochemical parameters of pancreatic and thyroid functions through toxicity studies. Fac. Agric. Ain Shams Univ. Res. Bull., 0 (1272): 1- 12.

El-Hennawy SI, Shama AI, Geabah NE and Sadek M (1980b): The effect of 3 herbicidal agents on biochemical parameters of liver function and cumulation lethal dose through toxicity studies. Fac. Agric. Ain Shams Univ. Res. Bull., 0 (1274): 1- 15.

El- Mahrouky F, Sanad AE and Fatma K (2001) :Effect of methomyl and camphor leaves ethanol extract on some transaminases enzymes and total protein in birds. J. Agric. Sci. Mansoura Univ., 26(10): 6437- 6450.

El-Missiry MA and Othman AL (1993): Influence of lannate on biochemical and hematological parameters in old rats. J. Egypt. Ger. Soc. Zool., 11(A), Comparative physiology, 219- 229.

El-Tarras A, Amal YS, Eweis E and Kandil MA (1995): Assessment of some toxicological and genetical parameters of imidacloprid on white mice. J. Agric. Sci. Mansoura Univ., 20(5): 2535- 2543.

Eman GEH, Samir AMZ and Abdel-Hamid BHR (1997): Biochemical and hematological effects of 8- Quinadldine dimethyl carbamate metholodide on albino rats. J. Egypt. Ger. Soc. Zool., 24(A) Comparative physiology, 119- 133.

Gluth G and Hanke W (1985) :A comparison of physiological changes in carp, Cyprinus carpio, induced by several pollutants at sublethal concentration. Ecotoxicol. And Environ. Safety, 9: 179- 188.

Gupta PK (1974): Malathion induced biochemical changes in rats. Ata. Pharmacol. Ectoxicol., 35: 191- 194.

Hare RS (1950) : Endogenous creatinine in serum and urine. In: Proc. Soc. Exp. Biol. (N. Y.) 74, pp. 148–151.

Hernandez T and Coulson R A (1967): Amino acid excretion in the alligator. Comp. Biochem. Physiol. 23, 775-784.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Khadre SEM (1988): The effect of experimentally induced inflammation on the blood pattern and hemopoietic organs of the teleost, Clarias Lazera. Bull. Inst. Oceanogr. Fish. ARE, 14,(14): 191- 203.

King EJ and Wootten IDP (1959) : Microanalysis in. Medical Biochemistry, p. 51. London: J. and A. Churchill

Leicht IW (1996): Imidacloprid a chloronicotinyl insecticides biological activity and agricultural significance. Pflanzenschutz-Nachrichten Bayer, 49: 71- 84.

Marie SM, Haggag AM and Ashraf AE (1998): Physiological and biochemical responses of the common carp, Cyprinus carpio, to an organophosphorous insecticide "Profenofos". Egypt J. Zool., 31: 279- 302.

Maxine M and Benjamin BS (1985): Outline of veterinary clinical pathology 3rd edition, Colorado state University, Printed in India at rakha printers PVD., New Delhi

Mitjavila S, Carrera G, Boigegrain RA and Deruche R (1981): Evaluation of the toxic risk of DDT in rat during accumulation. Arch. Environ. Contam. Toxicol., 10: 459- 469.

Parwinder SG, Kevin TP and David JS (2001): Neonicotinoid insecticides alter diapause behavior and survival of overwintering white grubs (Coleoptera: Scarabaeidae). Pest Manag. Sci. 57, 852-857.

Patyza S, Ziolo T and Nagorana B (1965): Effect of coumarin on histological and histochemical picture of parenchymatousorgans of rabbit. Annals, Univ. Mariae Curie, Sklodowska, Lublin, Pol. Sect. D.D. Med. Vert., 20: 75.

Rao KSP and Rao KVR (1984) : Changes in the tissue lipid profiles of fish (*Oreochromis mossambicus*) during methyl parathion toxicity - a time course study. Toxicology letters 21: 147- 153.

Richmond W (1973) : Preparation of properties of the cholesterol oxidase from nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin. Chem. 19: 1350- 1356.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Saleh F and El-Saify AA (1990) : Effect of methomyl and trichlorofon on body weight and some hematological parameters in two Egyptian birds. *J. Egypt. Ger. Soc. Zool.*, 1: 1- 13.

Sayed MA (2001) : Effect of imidacloprid, lead and their mixture on the hepatic esterase activity and metallothionein. *J. Agric. Sci. Mansouta Univ.*, 26(5): 3178- 3202.

Trinder P (1969): Determination of glucose in blood using glucose oxidase with an alternative oxygen. *Ann. Clin. Biochem.* 6:24-27

Uppal RP and Ahmed A (1977): Blood cholinesterase and serum transaminases in malathion toxicity in buffalo calves. *Indian J. Anim. Sci.* 47: 636-639.

Yamamoto I, Yabuta G, Tomizawa M, Saito T, Miyamoto T and Kagabu S (1995) : Molecular mechanism for selective toxicity of nicotinoids and neonicotinoids. *J. Pestic. Sci.* 20 (1995), pp. 33-40.

Young DS, Thomas DW, Friedman RB and Pestaner L C (1975): Effects of Drugs on Clinical Laboratory Tests. *Clin. Chem.* 21:5.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



PATHOLOGICAL EFFECT Of METALAXYL FUNGICIDE ON RABBIT'S KIDNEY AND URINARY BLADDER

Khadra M. Soliman*, Sawsan M. Jalalah** ; Afaf A El Ghawas* and Rawhia E Doghaim***

* Pathology Department ,Animal Health Research Institute, Dokki, Giza

** Pathology Department, College of Medicine, King Abdul-Aziz University, Jeddah

*** Pathology Department of, Faculty of Veterinary Medicine, Cairo University

ABSTRACT

Metalaxyl sprayed ration (MSR) was daily offered (contain 0.5 mg/kg) for NewZealand rabbits (young weaned age) to study the possible toxic effect of such low dose for 24 weeks. Rabbits were given the same MSR, revealed regular gradual decrease in level of Total protein and albumin . Transmission electron microscopy of kidney tissues were performed. Kidney function tests indicated marked renal failure represented by significant rise of creatinin and blood urea nitrogen. Histopathologically, glomerulonephritis accompanied by interstitial nephritis were common. Transmission electron microscopy revealed mesangial cell proliferation accompanied by increased amount of mesangial matrix . It was concluded that even using a very low dose of Metalaxyle, it induce toxic effect especially on kidneys

Key words: Histopathology, Metalaxyl, Rabbit and toxicity

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



INTRODUCTION

Metalaxyl is widely used as foliar sprays, seed dressing and soil treatment for protection against pathogenic fungi affecting the quality and shelf marketing duration of many freshly consumed vegetables as well as many fruits (**Kidd and James, 1991**). Post harvesting treatment is also widely used in grains. Fungicide metalaxyl is one of the common group of such products that was introduced since 1977; (**Ciba-Geigy, 1984**). Health hazard resulting from the abuse or over exposure is of common occurrence, mainly hepatic, renal, pulmonary gonadal or neurological damage (**Jorens and Schepens, 1993**). Renal failure was reported in underdeveloped population (**Caffarelli et al., 1999b;and Kaloyanova et al., 1999**). The literature review suggests a great need to increase awareness among people occupationally or environmentally exposed to pesticides about their potential negative influence on health of their children. (**Jurewicz J, and Hanke W.,2006**) Toxicity studies in humans, animals was given in addition to its persistence (long life)in the environment by (**Lin, and Garry ,2000**). Moreover, (**Demasia et al 2007**) found that metalaxyl induced in vitro micronucleus formation and sister chromatid exchange induction in human lymphocytes as well as in vivo micronucleus induction in the polychromatic erythrocytes of rat bone marrow, both separately or in combination with one another. Apoptosis and bax expression were observed in the hepatocytes of mice that were treated with metalaxyl (**Sakr et al 2011**). (**Dasgupta et al.2011**) reported that residues of buprofezin, chlorpyriphos, metalaxyl, and myclobutanil were detected in incurred grape and wine samples.

The aim of the present study:

- Morphological, histopathological examination of kidney and urinary bladder of rabbits consuming polluted food with metalaxyl fungicide that is currently and widely used in the field in an experimental animal model.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



- Estimation of kidney function test to evaluate the performance of essential detoxication and excretion mechanism.
- Electron Microscopical investigation of affected kidney tissue.

MATERIAL AND METHODS

Thirty two weaning male New Zealand rabbits age (45 days) were given 0.05 mg daily metalaxyl/kg sprayed ration (MSR) continuously along the time of experiment that extended up to 24 weeks. Rabbits were killed by slaughtering after collection of blood at 2, 4, 12 and 24 weeks.

Table (1): Number of control rabbits and those fed on MSR during the whole time of experiment.

Group	Dose	Period in weeks	No. of rabbits fed on MSR	No. of rabbits fed on balanced ration (control)
G1	0.05 mg/kg fed MSR	2	5	2
G2		4	5	2
G3		12	5	2
G4		24	7	4
Total		24	22	10

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Serum enzymes related to renal function, including urea nitrogen and creatinine were estimated according to **Kaneko and Cornelius (1971)**.

Statistical analysis according to **Parker,(1979)**.

Histopathological examination of kidneys urinary bladder sections stained with hematoxylin and eosin **Bancroft and Gamble(2002)**were done.

Transmission electron microscopic examination was done after fixation of tissue samples in 5% glutaraldehyde, semithin sections stained by toluidine blue then ultrasections were examined via photomicrographs. **(Hayat, 1989)**.

RESULTS

Biochemical findings:

Serum enzymes related to renal function, including urea nitrogen and creatinine were gradually increased during the experiment that extended for 24 weeks. In addition there was a remarkable decrease in serum total proteins as well as albumin in a dose time/related sequence. Remarkable increase in serum globulin. As presented in **Table (2) & Table(3)**.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Table (2): Mean values of some kidney function biochemical constituents in sera of rabbits treated orally with 0.05 mg metalaxyl / kg ration for 24 weeks.

Parameter	Time (week)							
	2 weeks		4 weeks		12 weeks		24 weeks	
	C	M	C	M	C	M	C	M
Urea nitrogen mg/dl	13.60 ±2.547	16.40 ±2.771	14.50 ±2.461	21.88 ±2.578	15.40 ±2.319	29.77 ±3.345*	17.90 ±3.381	54.60 ±10.013**
Creatinine mg/dl	0.76 ±0.212	0.773 ±0.237	0.80 ±0.254	1.36 ±0.196	0.84 ±0.151	1.78 ±0.177*	1.41 ±0.314	3.06 ±0.824**

C : Control group

M : Group fed on MSR

* : Significant

** : Highly significant

Mean ± SD

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Table (3): Mean values of some biochemical constituents in sera of rabbits treated orally with 0.05 mg metalexyl / kg ration for 24 weeks.

Parameter	Time (week)							
	2 weeks		4 weeks		12 weeks		24 weeks	
	C	M	C	M	C	M	C	M
Total protein g/dl	6.26 ±0.592	7.00 ±0.428	6.37 ±0.485	6.30 ±0.318	6.71 ±0.534	5.45 ±0.405*	7.04 ±0.502	4.99 ±0.666*
Albumin g/dl	3.84 ±0.581	3.87 ±0.421	4.03 ±0.485	3.04 ±0.203	4.36 ±0.568	2.12 ±0.522*	4.84 ±0.562	1.63 ±1.403**
Globulin g/dl	2.31 ±0.288	3.16 ±0.275	2.37 ±0.485	3.27 ±0.260	2.35 ±0.469	3.38 ±0.364*	2.20 ±0.537	3.36 ±0.433*

C : Control group

M : Group fed on MSR

* : Significant

** : Highly significant

Mean ± SD

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Pathology:

Gross appearance: -

At 2 and 4 weeks the kidneys showed congestion of the cortex. At 12 weeks congestion appeared at cortico-medullary junction. The cortical outer surface showed multiple depressed greyish foci of variable size. Such foci extended from the outer surface toward the deep cortex.

At 24 weeks multiple deep depressed foci were seen on the outer surface. Sagittal section showed congestion especially at calyces major and minor. Marked thickened wall of urinary bladder with streaks of congested blood vessels was detected.

Microscopic appearance: -

Kidneys at 2 weeks: -

The kidney had congestion of cortical and medullary blood vessels. Congestion of glomerular tuft, with infiltration of lymphocytes, increased cellularity of glomerular tuft were observed. Vacuolar degeneration and coagulative necrosis especially subcapsular tubules, some tubules had eosinophilic structureless proteinous cast. Lymphocytes invaded basement membrane and the epithelium lining of renal tubules, consequently, cellular cast was seen. Interstitial lymphocytic infiltration, extended radially from corticomedullary junction up to the outer surface. Some foci of cellular infiltration were extensive to replace focal area of damaged tubules at the vicinity of the glomeruli. The renal medulla showed multiple patches of lymphocytic infiltration with some plasma cells.

Kidneys at 4 weeks: -

Marked congestion of blood vessels, some glomeruli were collapsed while others had narrow Bowman's space. Other glomeruli had hypercellularity. The parietal epithelial lining the

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Bowman's capsule was prominently enlarged protruding into the lumen (Fig 1). Renal tubules underwent vacuolar degeneration and/or coagulative necrosis , infiltrated by lymphocytes invading its basement membrane (Fig 2). Eosinophilic proteinous casts could be detected. Dense lymphocytic infiltration with fibroblast proliferation, the latter was shown to replace some renal tubules of the cortex. The lymphocytic infiltration with active fibroblast giving wide streaks of collagen fibers proliferations extending from the deep cortex up to the capsule.

Kidneys after 12 weeks:

Marked congested blood vessels with haemorrhagic patches were seen with congestion of glomerular tuft. Congestion of medullary vessels could be observed . Most glomeruli had hypercellularity with mesangeal matrix proliferation (Fig 3).. Lymphocytic focal infiltration was scattered among the cortex. Wide patches of interstitial fibrosis were evident . Some renal tubules showed cystic dilatation while others showed vacuolar degeneration especially at the outer cortex. The basement membrane of renal tubules in the cortex and medullary segment of loop of Henle was thickened as evidenced by positive PAS reaction (Fig 3). Some renal tubules had cellular casts. Thickening in wall of most blood vessels and lamina elastica was disrupted. Consequently their lumen was narrow or stenosed (Fig 3). Homogenous globular eosinophilic structurless substance was shown inside the Bowman's space as well as tubular lumen (fig 4).

Kidneys after 24 weeks:

Renal cortex showed either focal glomerular atrophy, segmented glomerular tuft (Fig 5), or focal glomerular sclerosis. Some afferent arterioles were congested. Most blood vessels had thickened wall with swollen endothelial lining. Renal cortex had hemorrhagic patches. Sclerotic foci in renal medulla extend to the cortex(Fig 5), reached its capsule. Wide separation between renal tubules in medulla and cortex by the fibrous replacement especially in the outer zone of the

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



cortex . Marked thickening in basement membrane of renal tubules was confirmed by positive PAS reaction . Many tubules were, compressed, atrophied, others were cystically dilated. Hyaline, cellular or epithelial casts were constant finding. Coagulative necrosis was an outstanding change in proximal convoluted tubules. Some other tubules showed necrobiotic changes (Fig 5). Infiltration with mononuclear cells mainly lymphocytes, plasma cells and some eosinophils was observed.

Urinary bladder at 24 weeks: -

During postmortem examination of rabbits of this group urinary bladder was noticeably enlarged. On opening and evacuation, its wall was markedly thickened with corrugated inner surface. For this reason it was examined microscopically.

Microscopically, congestion with marked oedema were seen. Hyperplastic papillomatous folds were thrown into the lumen and covered by stratified transitional epithelium could be detected (Fig 6). Hyperplasia of covering epithelium in the form of ingrowing folds of connective tissue projecting into the lumen, which was branched several times (Fig 6). Epithelial covering of this papillomatous growth was observed as a transitional cell overgrowth (Fig 6). The lining epithelium was of granular vacuolated cytoplasm (Fig 6). Other cells were enlarged with hyperchromatic appearance,

Transmission Electron Microscopic Examination of kidney cortex:

E.M. Kidney after 12 weeks:

Glomerular EM revealed mesangial cell proliferation (Fig 7) accompanied by increased amount of mesangial matrix. Electron dense deposits were distributed among such matrix . Consequently collapsed capillary loop could be detected. Light electron dense multilamellated

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



bodies were detected inside the podocyte cell and mesangial matrix (Fig 8). Some erythrocytes were sometimes seen within the urinary space (Fig 9). Moderate fusion of podocyte's foot processes was more or less prominent in some cases .

Proximal convoluted tubules showed increased microvilli, many free ribosomes at the apical part of its cytoplasm as well as highly folded plasma membrane of tubular epithelial cells . Mitochondrial swelling was observed, with vacuolation of the matrix and disappearance of the cristae . some of them had electron dense lipoprotein deposits as well as deformity of mitochondrial shape. (Fig 10).

E.M. Kidney after 24 weeks :

The most prominent changes consisted of wrinkling of capillary basement membrane, fusion of podocyte's foot processes and proliferation of mesangial cells (Fig 11). Increased mesangial matrix was also detected in an extensive manner with electron dense deposits through it. Proximal convoluted tubules showed the so called "Dark cell-Light cell" phenomena (Fig12).

DISSCUSION

A- Biochemically:

Increased blood urea nitrogen and creatinine was known to be mainly and directly related to decreased glomerular filtration. The latter may be due to reduced renal functional units, the nephrons, hence accumulation of blood nitrogenous waste product of metabolism. Such loss of functional units is an accompaniment of nephrotoxicosis, (Hassan et al.; 1993, Takaori; 1993 Mohssen, 2001 and Selmanoglu et al., 2001).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



An increased level of blood urea nitrogen as well as creatinine was reported in the current study was directly related to the time up to the 24th week. Such data are considered highly specific to the efficiency of renal function performance as given by (**Selmanoglu et al., 2001; Ghoudhary et al., 2003 and Ali, 2004**).

A confirmatory findings were given by histopathological nephrotoxic changes. The latter were represented by two-fold changes. First the glomerular tuft was disrupted, as its mesangial cells were swollen, mesangial matrix was thickened, leading to impaired filtration. Second factor leading renal impairment was the tubular epithelial damage by the action of fungicide "Metalaxyl" or its toxic metabolite formed in the liver. Conclusively renal dysfunction is the final outcome, renal tubular damage coinciding with hepatic damage was shown to result of excess excretion of the metabolic waste product produced from hepatocytes (**Guarda et al; 1985 and Kim, et al 1997**).

Numerical loss of nephrons could be regarded as an additional factor. The latter was claimed to the marked fibrosis that occupy and replace great amount of renal parenchyma especially the cortex, thus reducing the number of functioning nephron. Histopathological investigation revealed collapsed glomeruli with thickened basement membrane and dilated tubules filled with eosinophilic casts these findings are coincides with that given by (**Milutinovic et al., 2003**). Nephrotoxicity has been attributed primarily to renal haemodynamic alterations caused by efferent arteriolar vasoconstriction. direct tubular injury is likely to contribute to nephrotoxicity (**Carvalho et al., 2003**).

B- Pathology :

Hypremia, is considered an essential criteria of acute inflammatory condition, as an accompaniment of both glomerular with tubular damage induced by the fungicide metalaxyl as well as its metabolic byproduct. The kidney cortex showed degeneration of renal tubules, atrophy

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



of glomeruli and intertubular leucocytic infiltration. It is suggested that the histological and histochemical alterations observed in kidney by fungicide may be mediated by depletion of antioxidants and elevation of lipid peroxidation (**Saber and Sumya 2012**). Excessive exposure to the maneb and zineb caused acute renal failure and nephrotic syndrome in agricultural workers and led to kidney damage (**Odermatt, 2004**).

Increased cellularity, mesangial proliferation and increased mesangial matrix could be attributed to a reactive proliferation that may compensate glomerular tuft destruction. This finding comes in an accordance with that given by (**Tuschiya et al., 2004**), who described panglomerular deposition of homogenous esinophilic globular structurless granules. This protein molecules could be considered an outcome of damage and leakage of glomerular filtrate, that let plasma protein, albumin, to pass and appear as protein casts.

C- Transmission electron microscopic:

A confirmatory results for glomerular mesangial cell proliferation was shown to appear in EM image in comparable large number than usual. Fusion and shortening of podocytes could be due to such toxic damage leading to impaired renal function. Fusion of podocyte's foot processes, with large amounts of debris in the Bowman's space. Low-density lamellar structures were present not only in the glomerular epithelium, basement membrane, mesangial matrix, parietal epithelium but also within the Bowman's space depend on the severity of the glomerular lesion (**Reena et al., 1999 and Tuschiya et al., 2004**). Haemorrhagic patches was seen in the cortex at early stages of the experiment, this is in agreement with that given by (**Luciano et al., 2004**) who stated that The response of kidneys acutely damaged by ischemic toxins is dominated by epithelial destruction and regeneration. That implicated by abnormalities in renal microcirculation, especially with regard to peritubular capillaries. Mostly nephrotoxicity was associated with tissue hypoxia, especially in degenerating tubules. This could be explained by the early glomerular damage, its increased cellularity by proliferating mesangial cells and

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



matrix, all of these are involved in hindrance to the flow of arterial blood from the related afferent arterioles. The resultant congestion may lead to marked dilatation of afferent arterioles or haemorrhage. Thickening of glomerular basement membrane along with tubular basement membrane was evident Meanwhile ,efferent arterioles coming out from affected glomeruli will suffer from ischemic condition. Tubular damage coincides well with those given by (**Yuan et al., 2003 and Luciano et al., 2004**).

Transmission EM image of proximal convoluted tubules revealed so called dark cell, light cell phenomena. (**Moutabarrik et al., 1992; Tanimoto et al., 1993 and Ghadially, 1996**) refered this phenomena to be almost an apoptotic change that affects single cells as a result of intoxication. In such phenomena dark cells are regarded as dead cells that has pyknotic nucleus, dehydrated shranked cytoplasm and removal by the neighboring healthy cells or tissue macrophages (**Moutabarrik et al.,1992**).

Urinary bladder mucosa was shown to became hyperplastic or papillomatous.In growing folds of mucosa supported by strands of connective cores that branch and rebranch toward the lumen was observed. Direct contact effect of the fungicide metalaxyl and toxicant waste product and metabolites on the surface epithelium of urinary bladder may be blamed in initiation of this hyperplastic or papillomatous growth.(**Brusick , 2005**).

CONCLUSIONS

1- Consumption of food containing even the permissible residual limit

up to 0.05 mg/kg is considered deleterious to essential detoxicating excretory organ, kidney.

2- Renal failure as a great threatening problem was also recorded.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



3- There is a suspicion for a cumulative effect of metalaxyl as its effect was more pronounced by elapse of time.

Figures Legend:

Fig. (1): Kidney of rabbit fed on MSR for 4 weeks showing hypercellularity of glomeruli. The parietal epithelial of Bowman's capsule is enlarged protruding into the lumen (arrow). H & E x 400

Fig. (2): Kidney of rabbit fed on MSR for 4 weeks showing vacuolar degeneration and coagulative necrosis of renal tubules infiltrated by mononuclear cells. H & E x 200

Fig. (3): Kidney of rabbit fed on MSR for 12 weeks showing glomeruli had hypercellularity with mesangeal matrix proliferation (arrow). PAS x 200

Fig. (4): Kidney of rabbit fed on MSR for 12 weeks showing globular esinophilic substance inside tubular lumen (arrow). H & E x 200

Fig. (5): Kidney of rabbit fed on MSR for 24 weeks showing wide patches of interstitial fibrosis and congestion of glomerular tuft. H & E x 200

Fig. (6): Urinary bladder of rabbit fed on MSR for 24 weeks showing hyperplasia of epithelium in the form of ingrowing folds, which is branched and transitional cell covering of the papillomatous growth. H & E x 200

Fig. (7): E.M. images of renal glomeruli after 12 weeks fed MSR showing mesangial cell proliferation (arrow). X 4000

Fig. (8): E.M. images of renal glomeruli after 12 weeks fed MSR showing electron dense multilamellated bodies (arrow) inside the podocyte cell and mesangial matrix. X 4000

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Fig. (9): E.M. images of renal glomeruli after 12 weeks fed MSR showing expansion of mesangial matrix and erythrocytes within the urinary space (arrow). X 4000

Fig. (10): E.M. images of renal proximal convoluted tubules after 12 weeks showing mitochondrial swelling, some had electron dense lipoprotein deposits (arrow). X 4000

Fig. (11): E.M. images of renal glomeruli after 24 weeks fed MSR showing highly proliferated mesangial cells and matrix (arrow). X 10,000

Fig. (12): E.M. images of renal glomeruli after 24 weeks fed MSR showing proximal convoluted tubules with dark cell-light cell phenomena. X 4000

REFERENCES

Ali, B.H. (2004): The effect of Nigella sativa oil on gentamicin nephrotoxicity in rats. Am. J. Chin. Med. Med., 32 (1): 49-55.

Bancroft , J.D. and Gamble , (2002): Theory and practice of histological technique. 6th Ed., Churchill, Livingston, New York, London, San Francisco, Tokyo

Brusick, D. (2005): Analysis of genotoxicity and the carcinogenic mode of action for ortho-phenylphenol. Environ Mol Mutagen. Jun;45(5):460-81.

Caffarelli ,V., Rapagnani, M.R., Letardi ,A. and Triolo, L. (1999a):
Pesticide residues in fruit and vegetable products and the carcinogenic risk for consumers
Rivista di Scienza dell Alimentazione , 28 (11): 11- 24.

Caffarelli , V., Rapagnani, M.R., Letardi, A., Triolo, L., Santaroni, P. and Lancia, (1999b):
Pesticide residues in horticultural products and carcinogenic risk for consumers. Proceedings of the XI symposium pesticide chemistry, Cremona, Italy, 11-15. September, 665-669.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Carvalho da Cost,a M., de Castro, I., Neto, A.L., Ferreira, A.T., Burdmann, E.A. and Yu, L, (2003): Cyclosporin A tubular effects contribute to nephrotoxicity: role for Ca²⁺ and Mg²⁺ ions. Nephrol. Dial. Transplant., Nov, 18 (11): 2262-8.

Ciba-Geigy Corporation (1984): MRID No. 00144371. Washington, DC 20460.

Dasgupta, S., Banerjee, K., Dhumal, K.N., Adsule, P.G. (2011): Optimization of detection conditions and single-laboratory validation of a multiresidue method for the determination of 135 pesticides and 25 organic pollutants in grapes and wine by gas chromatography time-of-flight mass spectrometry. J AOAC Int. 94(1):273-85

Demsia, G., Vlastos ,D., Goumenou ,M. and Matthopoulos, D.P. (2007) "Assessment of the genotoxicity of imidacloprid and metalaxyl in cultured human lymphocytes and rat bone-marrow" Mutat Res. Vol.634,no.1-2, pp.32-9.

Ghadially, F.N. (1996): Ultrastructural pathology of the cell and matrix. Fourth edition. Washnigton.

Ghoudhary, N., Shrma ,M., Verma, P. and Joshi, S.C. (2003): Hepato and nephrotoxicity in rat exposed to endosulfan. J. Environ. Biol., Jul, 24 (3): 305-8.

Guarda, F., Soffitti , M.G. and Nebbia (1985): Experimental and spontaneous toxicology and pathology of zinc ethylene dithiocarbamate in animals. Wiener Tierarztliche Monstschrift, 72 (5): 161-165.

Hassan, M.S., Rakha , G.M., Bashandy, M.M. and Hamouda, M.A.M (1993): Toxicity of Cupravit and Tilt in Albino Rats. Egypt. J. Comp. Pathol. Clin. Pathol., Vol. 6, No. 1 (March),

Hayat, M.A (1989): Principles and techniques of electron microscopy. Biological Applications. Third Edition, London: Butterworths.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Jorens, P.G. and Schepens, P.J. (1993): Human penta chlorophenol poisoning. *Hum. Exp. Toxicol.*, Nov., 12 (6): 479-95.

Jurewicz, J., Hanke, W. (2006): Exposure to pesticides and childhood cancer risk: has there been any progress in epidemiological studies? *Int J Occup Med Environ Health.* 19(3):152-69.

Kaloyanova, F., Ivanova Chemishanska, L., Zaykov ,H.R., Baynova, A., Mihaylova, A. (1999): Toxicological evaluation of agromet metalaxyl preparation. *J. Hyg. Epidemiol. Microbiol. Immunol.*, 35 (4): 375-382.

Kaneko ,J.J and Cornelius, C.E (1971): Clinical Biochemistry of Domestic animals, Second Edition Academic Press New York, London, 1971.

Kidd, H. and James, D.R (1991): The Agrochemicals Handbook, Third Edition. Royal Society of Chemistry Information services, Cambridge, UK.

Kim, H.S., Cha ,S.H , Abraham, D.G, Cooper, A.J.and Endou, H. (1997): Intranephron distribution of cystein s-conjugate beta-lyase activity and its implication for hexachloro-1,3-butadiene-induced nephrotoxicity in rats. *Arch. Toxicol.*, 71 (3): 131-41.

Lin, N. and Garry, V.F. (2000): In vitro studies of cellular and molecular developmental toxicity of adjuvants, herbicides, and fungicides commonly used in Red River Valley, Minnesota. *J Toxicol Environ Health A.* Jul 28; 60(6):423-39.

Luciano, M.N., da Silva , (2004): Experimental evidence for a direct cytotoxicity of *Loxosceles intermedia* (brown spider) venom in renal tissue. *J. Histochem. Cytochem.*, Apr, 52 (4): 455-67.

Milutinovic, A., Zivin, M., Zorc-Pleskovic, R. and Sedmak, B. (2003): Nephrotoxic effects of chronic administration of microcystins-LR and -YR. *Toxicon.*, Sep, 42 (3): 281-8.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Mohssen, M. (2001): Biochemical and Histopathological Changes in Serum Creatinine and Kidney Induced by Inhalation of Thimet (Phorate) in Male Swiss Albino Mouse, Mus Musculus. Environmental Research [Environ. Res.]. Vol. 87, no. 1, pp. 31-36. Sep.

Moutabbarik, A., Ishibashi, M. and Kameoka, H., (1992): In vitro FK506 Kidney tubular cell toxicity. Transpl. Int., 5 Suppl 1: S87-92.

Odermatt, A. (2004): Corticosteroid-dependent hypertension. Environmental influences. Swiss Med. Wkly., 134: 4–13

Parker , (1979): Introductory Statistics for Biology 2nd Ed. Arnold, London.

Reena-Kackar, Srivastava, M .K., Raizada, R. B and Kackar, R. (1999): Assessment of toxicological effects of mancozebin male rats after chronic exposure. Ind . J. Exper. Biol., 34 (6): 553-559.

Saber, A., Sakr and Somya ,Y. Shalaby (2012): Metiram-induced histological and histochemical alterations in Liver and kidney of pregnant mice Life Science Journal, 2012; 9(1):71-76.

Sakr , S.A., Lamfon, H.A. and Essawy ,A.E. (2011): Ginger (*Zingiber officinale*) extract ameliorates metalaxyl fungicide induced nephrotoxicity in albino mice. Afr J Pharm Pharmacol 5: 104-112

Selmanoglu, G., Barlas, N, ,Songur ,S. and Kockaya, E.A. (2001): Carbendazim-induced haematological, biochemical and histopathological changes to the liver and kidney of male rats. Hum. Exp. Toxicol., Dec., 20 (12): 625-30.

Takaori, H. (1993): Thiophanate-methyl combined chronic toxicity/oncogenicity study in rats. Unpublished report no.RD-9327 from Nisso Institute for Life Sciences, Kanagawa, Japan. Submitted to WHO by Nippon Soda Co. Ltd, Tokyo, Japan.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Tanimoto, A., Hamada, T. and Koid ,O. (1993): Cell death and regeneration of proximal tubular cells in rats with subchronic cadmium intoxication. Toxicol. Pathol., 21-41.

Tuschiya, N., Matsushima, S., Takasu, N., Kyokawa, Y. and Torii, M. (2004): Glomerular clacification induced by bolus injection with dibasic sodium phosphate solution in spraguedawley rats. Toxicol. Pathol. Jul-Aug, 32 (4): 408-12.

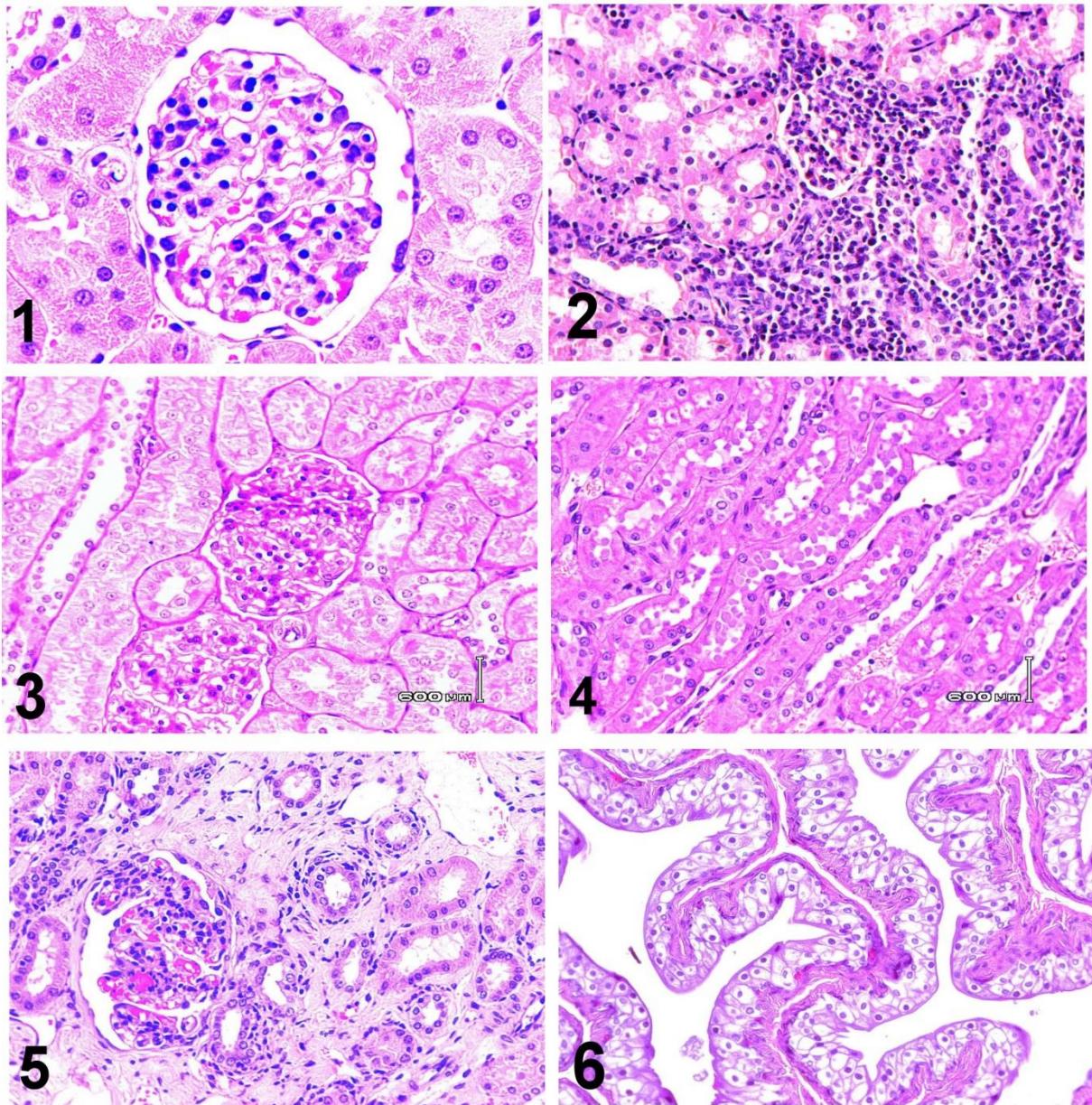
Yuan ,H.T., Li ,X.Z., Pitera, J.E., Long ,D.A. and Woolf, A.S. (2003): Peritubular capillary loss after mouse acute nephrotoxicity correlates with down-regulation of vascular endothelial growth factor-A and hypoxia-inducible factor-1 alpha. Am. J. Pathol., Dec, 163 (6): 2289-301.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

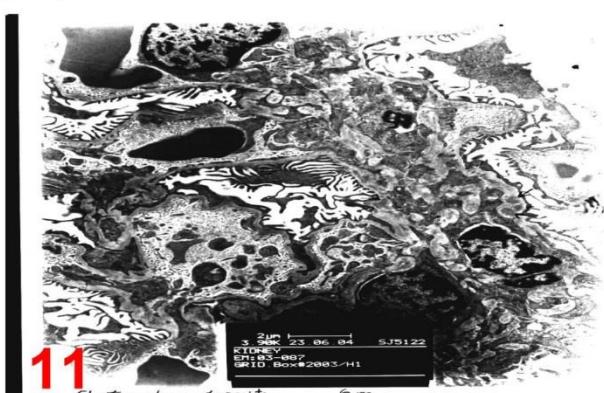
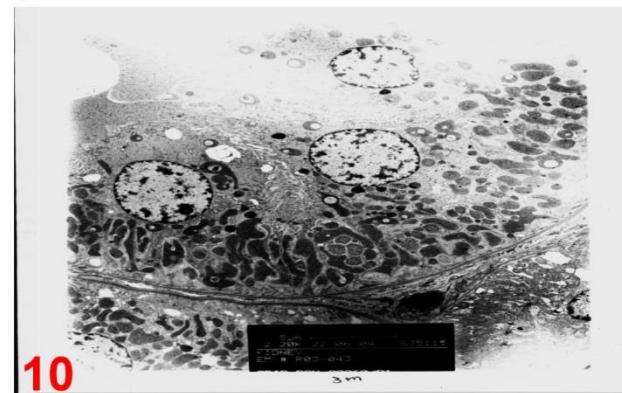
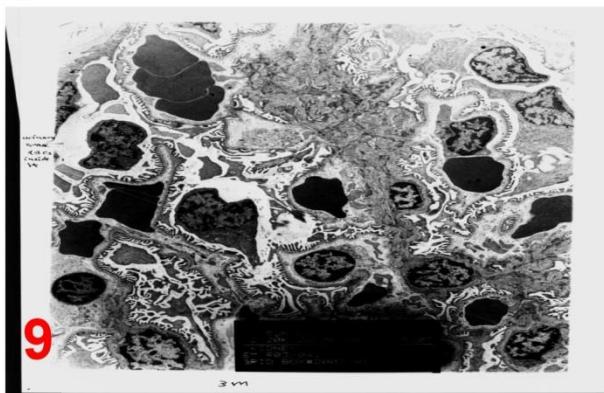


2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



TOXIC EFFECTS OF PROFENOFOS IN ALBINO RATS

Rania Abd El-Al A. H. *; Nariman A. Rahmy**; Randa A. Hassan** and El-araby E. K. M. ***

*Pharmacological Dept- National Organization For Drug, Control and Research (NODCAR)

** Pathology Dept-Animal Health Research Institute.

***Biochemistry Dept- Animal Health Research Institute.

ABSTRACT

The study aimed To compare between subacute and subchronic exposure to equitoxic doses (1/4 and 1/8 Of LD₅₀) of the two end-use products (EUP) of Profenofos (P) pesticide, from different manufactures, (Selecrone (Ps) and Cord (Pc)) on some biochemical indices and pathological feature of internal organs in male albino rats. One hundred male albino rats were divided into six groups. first and fourth groups were received tap water as a control group. Second and third groups were orally administered with (Ps) and (Pc) at the dose of 46.3 and 44.62 mg/kg body weight per day, respectively, (4 doses/week) for 28 days (subacute treatment). Fifth and sixth groups dosed with (Ps) and (Pc) at the doses of 23.14 and 22.31 mg/kg body weight per day, respectively, (four doses/week) for 60 days (Subchronic treatment). Liver and kidney functions and pathological features of internal organs (liver, kidney, lung and brain) were examined. Treatments with (Ps) and (Pc) at the dose of 1/4 LD₅₀ for 28 days revealed significant decrease in plasma AchE activity, while an elevation in the activity of AST and ALT enzymes occurred. In addition, Pc-treated group exhibited significant increase in total protein and albumin levels in comparison with the control group. Moreover, Ps-treated group showed significant increase in albumin and urea levels. Significant decrease in AchE activity was observed in animals treated

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



with (Ps) and (Pc) at the dose of 1/8 LD₅₀ for 60 days. On the contrary, (Ps) and (Pc) compounds did not alter AST and ALT activities, total protein, albumin, urea and creatine levels, except, AST activity and creatinine level were increased in Pc-treated group. Pathological studies in case of Selecrone revealed focal necrosis of hepatocytes with inflammatory cells aggregation in portal area in liver, glomeular atrophy with focal hemorrhagic areas in corticomedullary junction in kidney, Peribronchiolar lymphoid hyperplasia associated with collapse and emphysema of air alveoli in lung and focal gliosis and hemorrhage in cerebellum in brain. In cord, pathological alterations showed perivascular inflammatory cells infiltration with granular degeneration of hepatocytes in liver, cortical inflammatory cells infiltration in kidney, emphysema of the air alveoli in lung and focal gliosis in the cerebral tissue in brain. It is concluded that both (Ps) and (Pc) caused biochemical and pathological abnormalities of examined organs. The effects of (Ps) was significant than (Pc) on liver, kidney, lung and brain tissues.

Key words: Profenofos (P), Selecrone (Ps), Cord (Pc), Rats, transaminase, Total protein, urea, creatinine, histopathology, liver, kidney, lung, brain.

INTRODUCTION

Pesticides are widely used throughout the world in agriculture to protect crops and in public health to control diseases transmitted by vectors or intermediate hosts (**Farrag and Shalby, 2007**).

Organophosphorus insecticide is among the most frequently used pesticides. They are used in agriculture, forestry, horticulture, public health and the house. Organophosphates are well resorbed after uptake via the oral, dermal or inhalation route and are rapidly metabolized in the human body. In general, 90% of the compounds are excreted within 6-24h after oral uptake (**Leng et al., 1997a, Leng et al., 1997b and Oluah, 1998**).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Profenofos Organophosphorus insecticide is a widely used in Egypt for the control of various caterpillars, white fly and mites on cotton and vegetable crops (**Mikhail et al., 1979 and Hanafy et al., 1991**).

Zidan et al., 1998 stated that the wide use of insecticides in the agriculture is really threatening the health of consumers, either individually or in different combinations, although improved the quantity of the harvests, its quality regarding the insecticides (residues) in fruits, leaves or any other part of the plant .

Toxicity assessment revealed that cholinesterase is the most sensitive biomarker for male rats, while uric acid is the most sensitive biomarker for female rats (**Greisha et al., 2011**).

One-tenth of median lethal dose of Profenofos mostly affected on erythrocytes counts, hemoglobin levels and alkaline phosphatase activity. That would suggest that the tested compound have an inhibitory action on haemopiesis (**Shalby, 2006**).

El-Kashoury et al. (2006) recorded reduction in RBCs, WBCs and MCHC% in two treated groups of male albino rats orally dosed by 1/8 and 1/4 and LD50 of Profenofos, while decreased hemoglobin was significant in low dosed group. Also they added that testosterone was elevated significantly in high dosed group, while significant hypercholesterolemia and high levels of triiodothyronine (T3) were noticed in group received low dose.

Short-term administration of Profenofos (Ps) increased thyroxin in comparison with that of control and Profenofos (Pc) –treated rats. On the other hand, the short and long term effects of both treatments decreased triiodothyronine in comparison with control. However, the short and long term effects of both treatments increased triglycerides, high-density lipoprotein on comparison with those of control (**El-Kashoury and El-far, 2004**). **Hammam and Abd El-Mottaleb (2007)** recorded that Profenofos exhibited histopathological change in liver, kidney, spleen and testes.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Early studies were reported by El-Kashoury (2009), who investigate the effect of profenofos (Ps), at the dose of 1/8 LD50, 23.14 mg/kg b.w, Per OS. for 60 days, on the key enzymes of fertility and the concentration of micro elements in testicular tissues in male albino rats. Results showed significant decrease in testes and epididymus weights, as well as, reduction in sperm count was recorded in cauda epididymus, associated with decrease motility. Moreover, total protein exhibited an elevation, while there was decrease in the activities of alkaline and acid phosphatase (ALP and ACP) and lactate dehydrogenase (LDH). Moreover, the authors added that a sharp augmentation in the elements; copper (Cu), zinc (Zn), iron (Fe) and selenium (Se) was observed.

The current study was conducted to compare the subacute and Subchronic toxicity effects of two commercial products of Profenofos, one imported (Selecrone, Ps) and the second is produced locally (cord, Pc) on biochemical and histopathological features of vital body organs in adult albino rats.

MATERIALS AND METHODS

1-Expermintal animals

Experiments were performed on one hundred adult male albino rats (weighing 150 ± 10 g each) obtained from the farm of general organization of serum and vaccine (Helwan farm). Animals were allowed to be acclimatized to laboratory conditions (room temperature 20 ± 5 °C and 12:12 light : Dark) for a minimum of 2 weeks prior to the experiment and kept on a balanced diet and water ad libitum.

2-Experiment materials

Two end-use products of Profenofos pesticide, (an Organophosphorus group;"Ops"), were used in the present study:

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



First: Profenofos; trade name: Selecrone (Ps) obtained from Syngenta Comp., it used as insecticide.

Second: Profenofos; trade name: Cord (Pc) obtained from El-HELB Comp., it used as insecticide. Both Ps and Pc contain the same active ingredient 72% of Profenofos.

The oral LD₅₀ of Profenofos; Ps and Pc were 185.13 and 178.459 mg/kg b.w., respectively, according to **Weil (1952)**. Profenofos 72% EC. (Ps and Pc) were emulsified in water immediately before use and orally administered to animals by esophageal intubation (per OS.).

3-Experimental design.

3.1. subacute toxicity study:

Fifty rats were divided randomly into three comparable groups; first group (n=10) was kept as a control group and received the vehicle only (tap water). Second and third groups (n=20) were orally dosed (per OS.) for 28 days with Profenofos (Ps) and Profenofos (Pc) at 46.3 and 44.62 mg/kg b.w, respectively (4 doses/week). Doses represent 1/4 LD₅₀ of Profenofos (Ps and Pc, respectively).

3.2. Subchronic toxicity study:

Fifty rats were divided into three groups; first group (n=10) as control one. Second and third groups (n=20) were orally dosed for 60 days with profenofos (Ps &Pc) at 23.14 and 22.31 mg/kg b.w, respectively (4 doses/week). Doses represent 1/8 LD₅₀ of both two compounds (Ps & Pc) respectively.

Clinical signs were monitored daily throughout the two experiments (Subacute and Subchronic).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Sampling:

At the end of experimental period (28 and 60 days), all animals were anesthetized with ether and blood samples were collected from orbital sinus vein by heparinized capillary tubes into clean, dry Ependorf tubes contained Lithium heparin as anticoagulant according to Schalm (1986). Blood samples were centrifuged at 3600 rpm for 15 minutes in a refrigerated centrifuge to separate plasma. Plasma samples were kept frozen till the different assays were carried out. Four rats from each group were sacrificed and examined for post mortem lesions. A tissue specimen from liver, kidneys, lungs and brain were fixed in 10% neutral buffered formalin. Sections of 4-6 microns thickness were prepared and stained Haematoxylin and Eosin (**Bancroft and Gamble, 2002**).

Biochemical assays:

The following biochemical parameters were estimated according the corresponding authors. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to **Reitman and Frankel (1957)**; total protein "TP" (**Weichselbaum, 1946**); albumin (**Dumas et al., 1971**); acetylcholinesterase "AchE" (**Ellman et al., 1961**); urea (**Fawcett and Scott, 1960**) and creatinine (**Siest et al., 1985**).

4- Statistical analysis:

All data subjected to statistical analysis according to the procedure reported by **Snedecor and Cochran (1967)**.

RESULTS

1-Clinical symptoms:

A-Subacute treatment: Treated rats with Ps exhibited noticeable signs of intoxication, sedation and paralysis, gastrointestinal signs (vomiting and diarrhea). Moreover, mortality rats was 70%.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Meanwhile, rats treated with Pc compound didn't exhibited any signs of intoxication although 40% mortality occurred.

B- Subchronic treatment: Sedation was clearly observed and lose stool. The mortality rate reached 40% in the Ps group and 60% in Pc group.

2-Biochemical Analysis.

A-Subacute toxicity study:

Table (1) illustrated biochemical values of subacute toxicity of PS (Selecrone) and PC (Cord) in treated groups.

Acetyl cholinesterase activity showed high significant reduction in both tested compounds especially with Ps group about (75%) as compared to control group.

Aspartate amino-transferase (AST) revealed high significant increase in both tested compounds as compared to control group. Alanine aminotransferase (ALT) showed high significant increase in Ps treated group and significant rise in Pc-treated group as compared to control group.

Total protein (TP) was significantly increased with administration of Pc group, comparable to control group. Albumin (Alb) proved significant rise in Ps-treated rats, while Pc-treated group revealed a highly significant increase in comparison to control group.

Urea values recorded moderate significant increase in Ps-treated group compared to control group, while creatinine levels were within normal values recorded in control group.

B-Subchronic toxicity study:

Table (2) illustrate the biochemical values of Subchronic toxicity of PS (Selecrone) and PC (Cord) -treated groups.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Acetyl cholinesterase activity was significantly inhibited ($P \leq 0.01$). Maximum decrease about (65%) occurred in Ps-treated group in comparison with the control group.

Aspartate amino-transferase (AST) showed high significant increase in Pc-treated rats only, while Alanine amino-transferase, total protein and albumin were within normal values recorded in control group.

Urea was within normal levels recorded in control group, while creatinine revealed significant increase in Pc-treated group in comparison with the control group.

3-Histopathological Results

A-Subacute toxicity study:

I-Selecrone-treated group (1/4 dose):

Liver showed severe dilatation of the central and portal veins and sinusoids together with focal mononuclear leucocytic inflammatory cells aggregation in the portal area as well as periductal inflammatory cells infiltration with fibroblastic proliferation surrounding the bile ducts (fig.1). There was focal necrotic area in the hepatic tissue (fig.2,3). The kidneys revealed dilated engorged blood vessels and glomerular atrophy (fig.4) Dilated blood vessels with focal hemorrhagic area were seen in corticomedullary junction (fig.5). Lungs showed Peribronchiolar lymphoid hyperplasia associated with collapse and emphysema of the air alveoli .The Brain denoted dilatation in the cerebral blood capillaries with swelling of their lining endothelium (fig.6). Focal gliosis (fig.7) and hemorrhage in the subarachnoid meningeal tissue of the cerebellum were also detected (fig.8).

II-Cord-treated group (1/4 LD₅₀):

Liver Showed perivascular mononuclear leucocytic inflammatory cells infiltration associated with granular degeneration in the hepatocytes and diffuse kupffer cells hypertrophy allover the

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



hepatic tissue (fig.9). The kidney revealed focal mononuclear leucocytic inflammatory cells infiltration and dilatation in the blood vessels inbetween the renal tubules at the cortical portion (fig.10). Lung denoted Emphysema in the air alveoli (Fig.11). The Brain revealed focal gliosis in the cerebral tissue (fig.12).

B-Subchronic toxicity study:

I-Selecrone-treated group (1/8 LD50).

Liver showed few mononuclear leucocytic inflammatory cells infiltration in the portal area. The Kidney showed no alteration observed. Lung revealed Peribronchiolar lymphoid hyperplasia associated with collapse and emphysema of the air alveoli (fig.13). Brain showed Hemorrhage in the subarachnoid meningeal area and axonal degeneration (fig.14).

II-Cord-treated group (1/8 LD50):

Liver revealed dilatation in the central vein and sinusoids (fig.15), associated with inflammatory cells infiltration and dilatation in the portal vein in the portal area. Kidney showed dilatation in the cortical blood vessels (fig.16). In Lung Peribronchiolar and perialveolar blood capillaries were dilated and engorged with blood (fig.17). Brain showed focal gliosis in the cerebral tissue (fig.18).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Table (1): Effect of subacute toxicity of Profenofos (Ps) and Profenofos (Pc) (1/4 LD50) on biochemical parameters in adult male albino rats for 28 days.

parameter	Experimental groups *		
	Control	"Selecrone" (Ps)	"Cord" (Pc)
(AchE) U/L	268.36 ± 12.90	68.42±15.78***	166.18±21.57**
(AST) U/ml	49.85 ± 4.76	83.43±5.46***	79.37±4.29***
(ALT) U/ml	7.39 ± 0.94	13.99±0.88***	10.26±0.88*
TP (g/dl)	5.47 ± 0.19	5.96±2.9	6.31±0.11*
Alb.(g/dl)	2.16 ± 0.14	2.57±0.06*	3.05±0.03***
Urea(mg/dl)	37.96 ± 0.62	50.58±3.41**	36.33±0.79
creatinine(mg/dl)	0.752 ± 0.05	0.711±0.05	0.73±0.01

Values represent the mean ± SE (n=6) (Student's "t"-test)

*significant at P<0.05 ** significant at P<0.01 *** significant at P<0.001

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Table (2): Effect of subchronic toxicity of Profenofos (Ps) and Profenofos (Pc), (1/8 LD50), on biochemical parameters in adult male albino rats, for 60 days.

parameter	Experimental groups *		
	Control	"Selecrone" (Ps)	"Cord" (Pc)
(AChE) U/L	239.49±24.90	83.09±24.49**	121.21±13.36**
(AST) U/ml	57.49±2.24	55.26±0.61	68.12±3.6***
(ALT) U/ml	9.26±0.68	10.89±0.84	7.79±0.79
TP (g/dl)	6.56±0.21	7.00±0.28	6.09±0.23
Alb.(g/dl)	2.63±0.08	3.11±0.24	2.52±0.05
Urea(mg/dl)	49.95±4.16	53.74±2.74	49.26±3.59
creatinine(mg/dl)	0.770±0.03	0.730±0.05	0.90±0.01**

Values represent the mean ± SE (n=6) (Student's "t"-test)

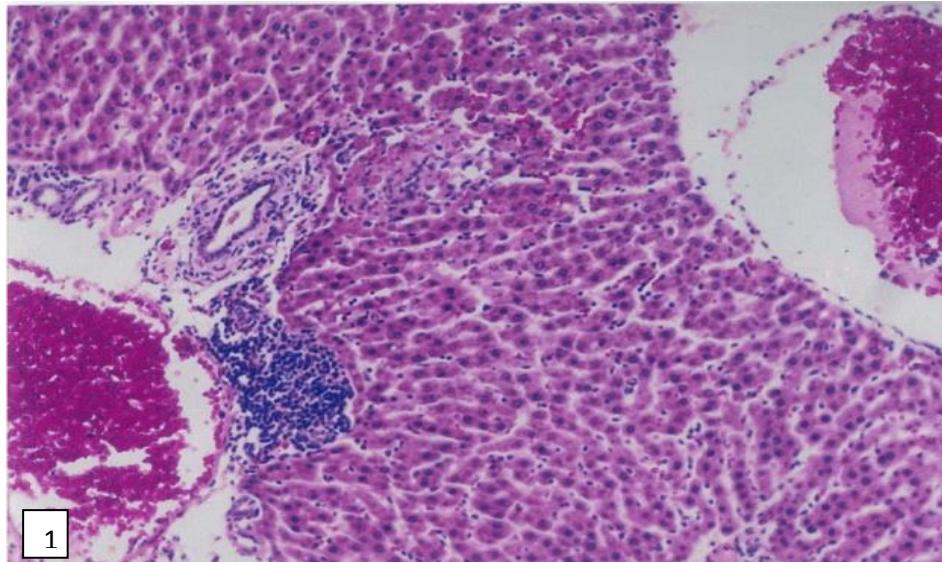
** significant at P<0.01 *** significant at P<0.001

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Fig(1): liver of rat treated by Selecrone (1/4 LD₅₀) showing sever dilatation of central portal veins as well as sinusoids with focal mononuclear leucocytic inflammatory cells aggregation in the portal area and periductal inflammatory cells infiltration and fibroblastic cells proliferation of bile duct (H &E X40).

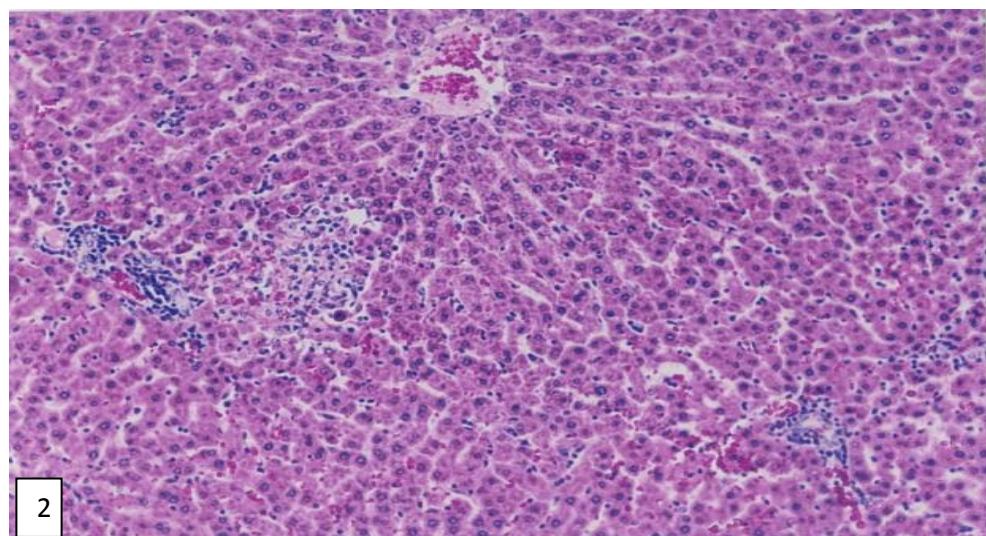


Fig (2): liver of rat treated by Selecrone (1/4 LD₅₀) showing focal necrosed area of the hepatic tissue (H &E X40).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

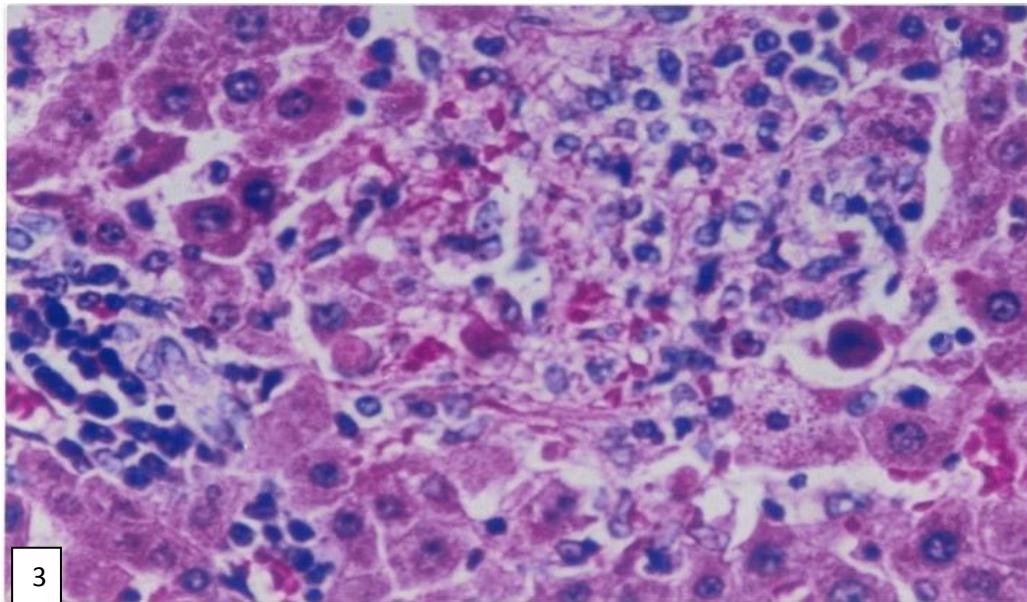


Fig (3): liver of rat treated by Selecrone (1/4 LD₅₀) showing focal necrosed area of the hepatic tissue by high magnification (H & E X160).

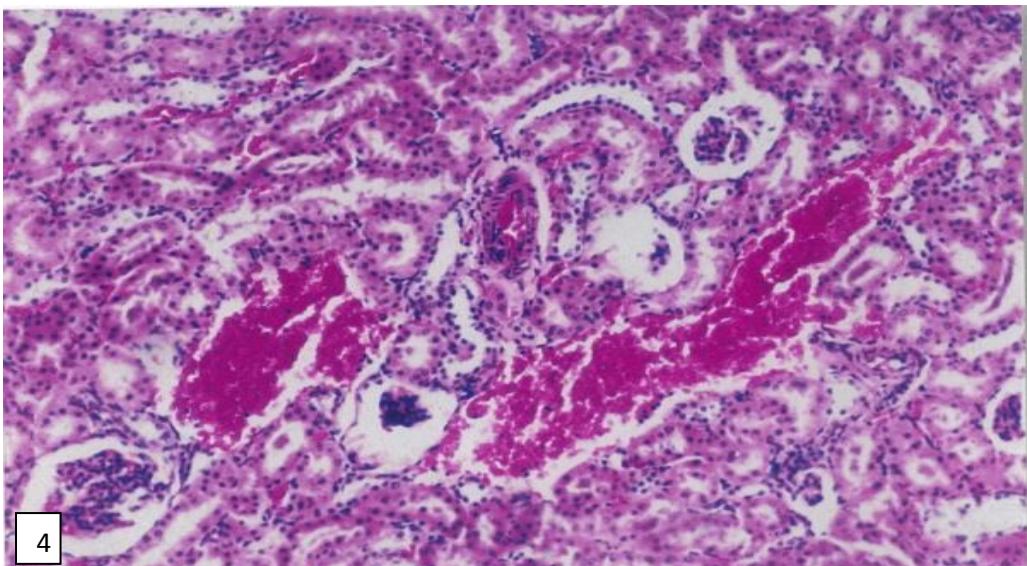


Fig (4): kidney of rat treated by Selecrone (1/4 LD₅₀) showing dilated and engored hyperemic blood vessels and glomeular atrophy (H&E X40).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

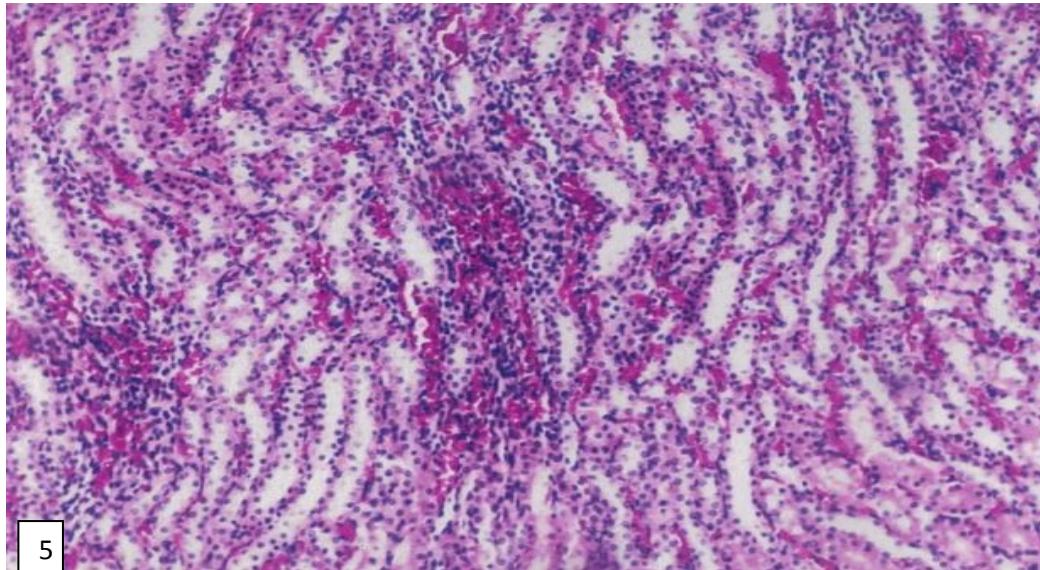


Fig (5): kidney of rat treated by Selecrone (1/4 LD50) showing dilated blood vessels and focal hemorrhagic areas in the corticomedullary portion (H&E X40).

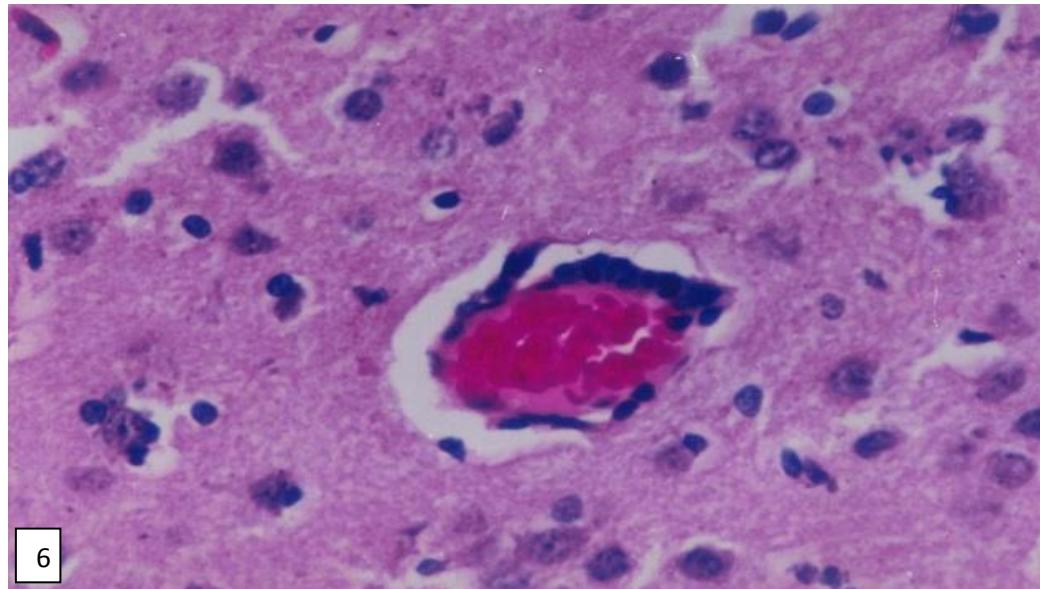


Fig (6): Brain of rat treated by Selecrone (1/4 LD50) showing dilated and engorged cerebral blood capillaries with swelling and proliferation of the lining endothelium (H&E X160).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

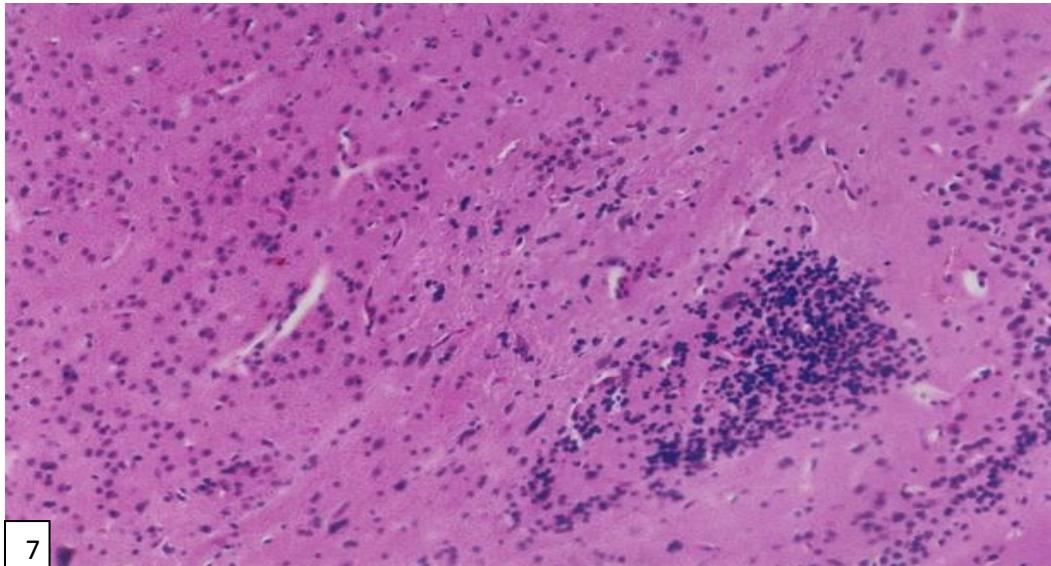


Fig (7): Brain of rat treated by Selecrone (1/4 LD₅₀) showing focal cerebral gliosis (H&E X40).

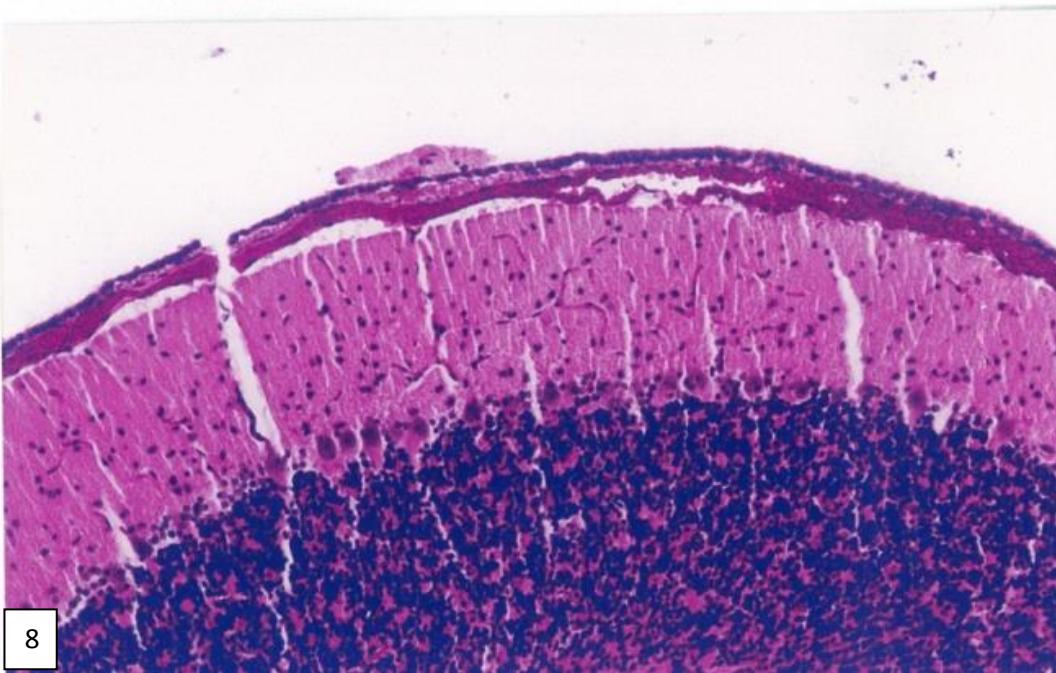


Fig (8): Brain of rat treated by Selecrone (1/4 LD₅₀) showing subarachnoid meningeal hemorrhage in the cerebellum (H&E X40).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

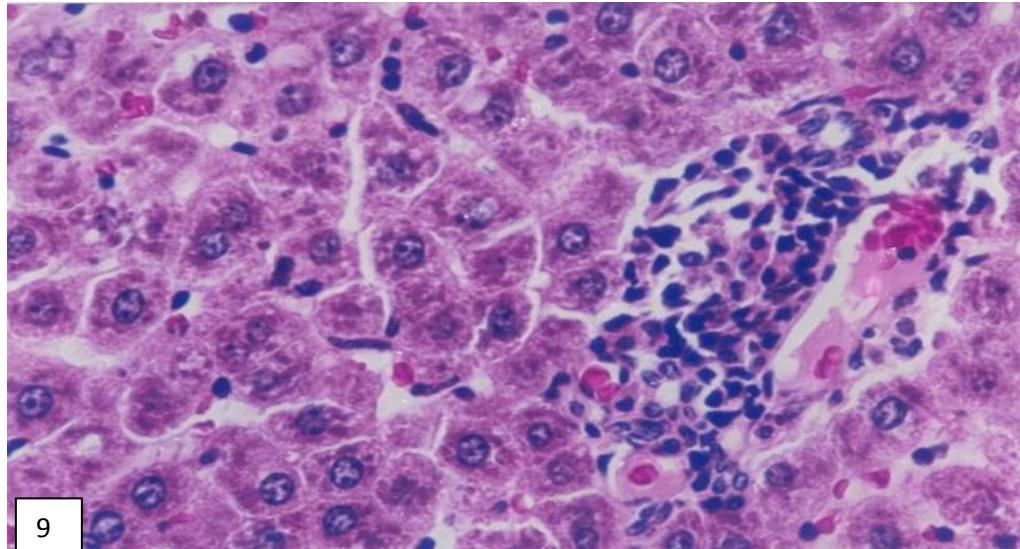


Fig (9): liver of rat treated by cord (1/4 LD₅₀) showing perivascular mononuclear leucocytic infiltration with granular degeneration in the hepatocytes as well as kupffer cells hypertrophy (H&E X160).

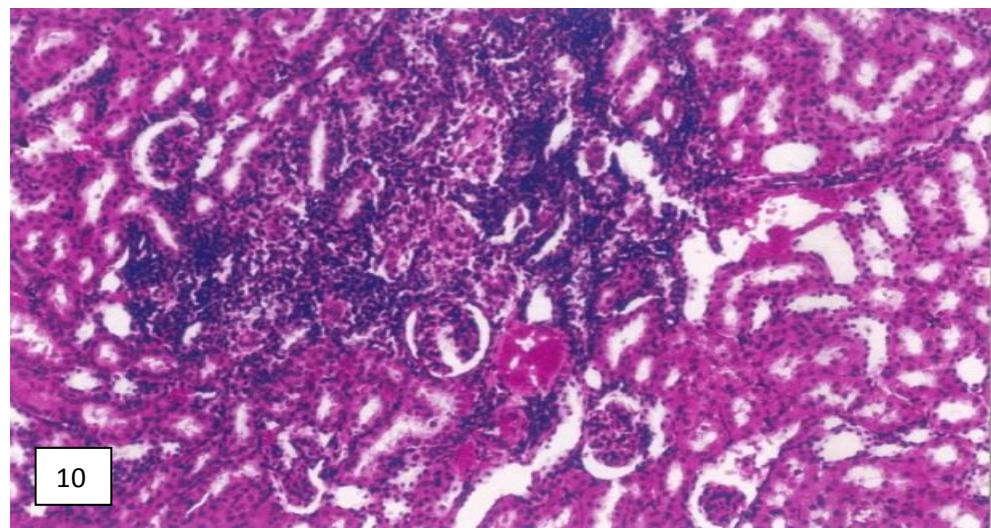


Fig (10): kidney of rat treated by cord (1/4 LD₅₀) showing focal cortical mononuclear leucocytic infiltration with dilated blood vessels (H&E X40).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

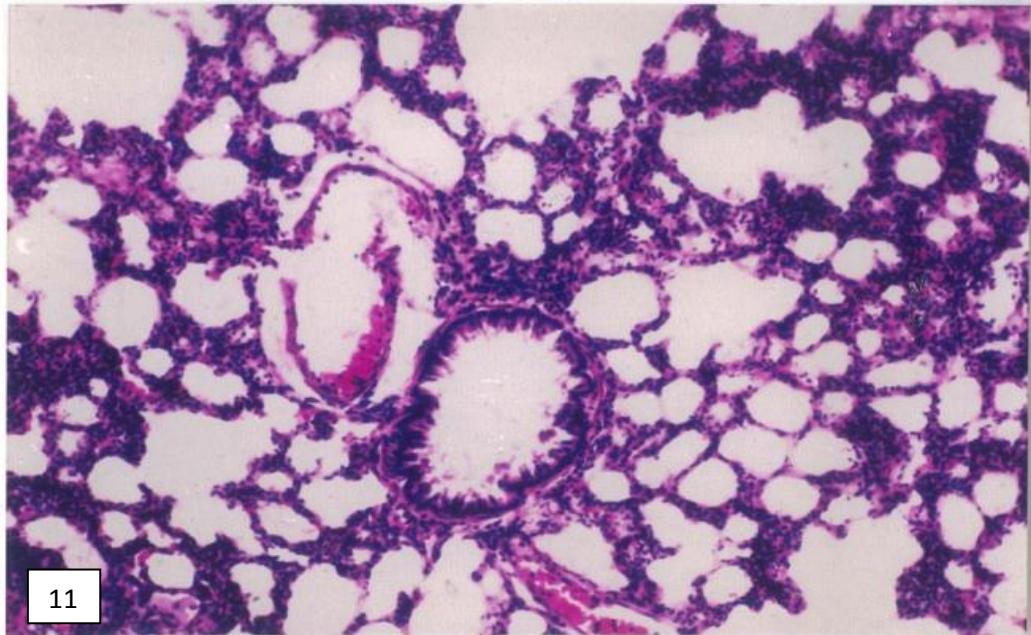


Fig (11): lung of rat treated by cord (1/4 LD50) showing emphysema of the air alveoli (H&E X40).

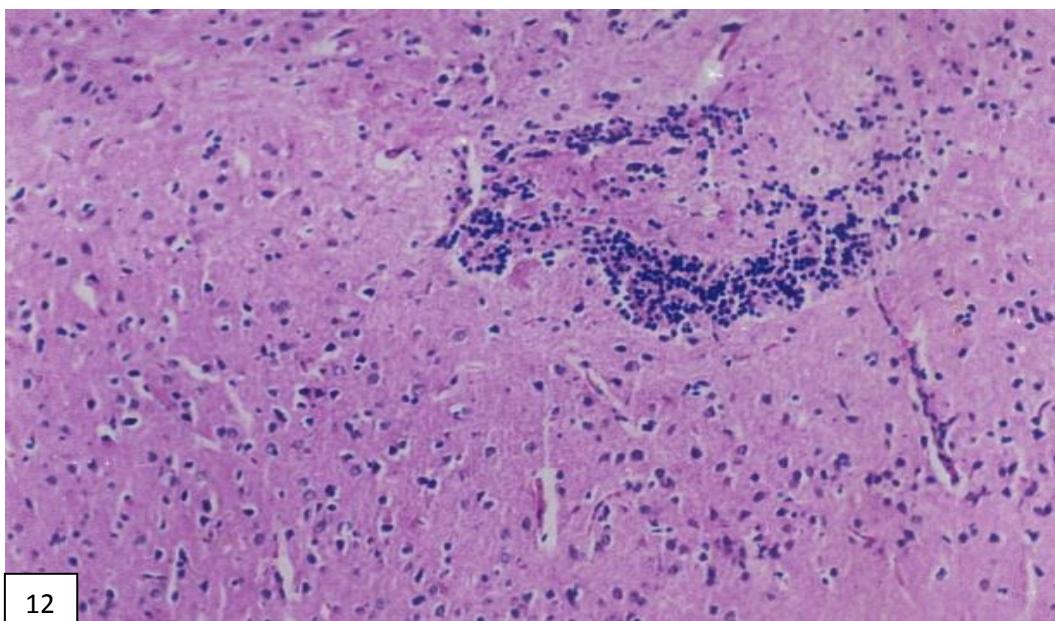


Fig (12): Brain of rat treated by cord (1/4 LD50) showing focal cerebral gliosis. (H&E X40).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

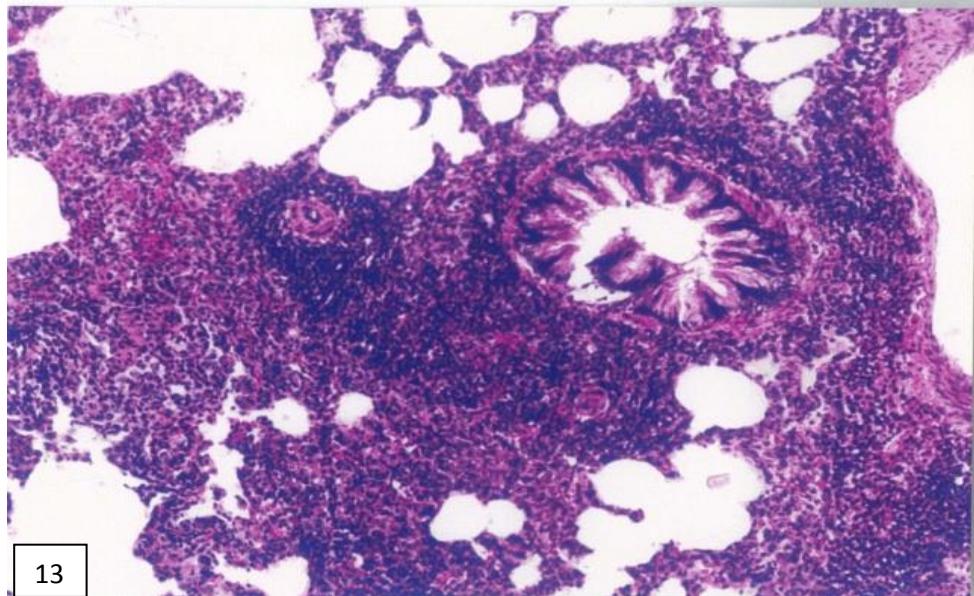


Fig (13): lung of rat treated by Selecrone (1/8 LD50) showing Peribronchiolar lymphoid hyperplasia with collapse and emphysema of the air alveoli (H&E X40).

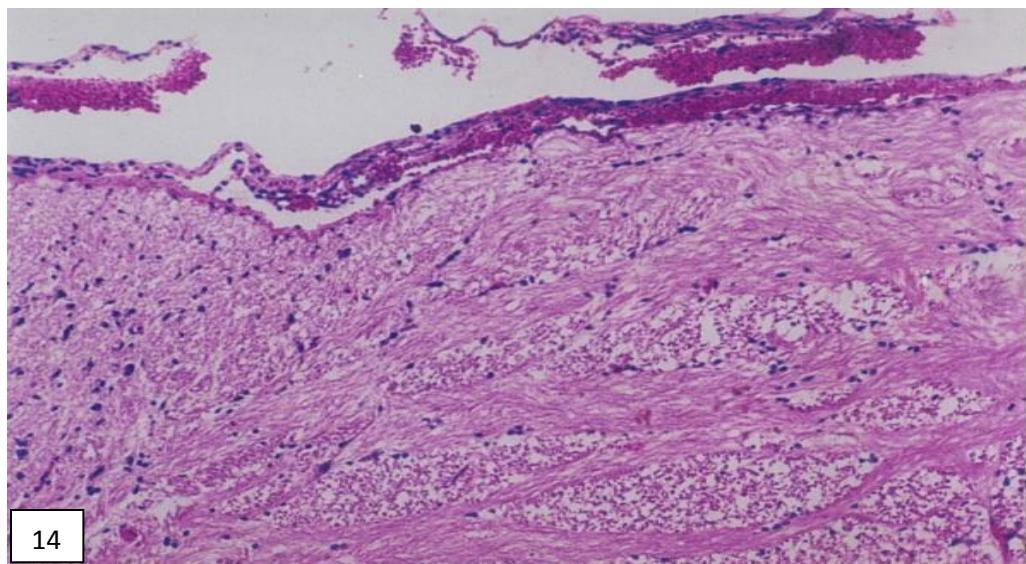


Fig (14): Brain of rat treated by Selecrone (1/8 LD50) showing subarachnoid meningeal hemorrhages and axonal degeneration (H&E X40).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

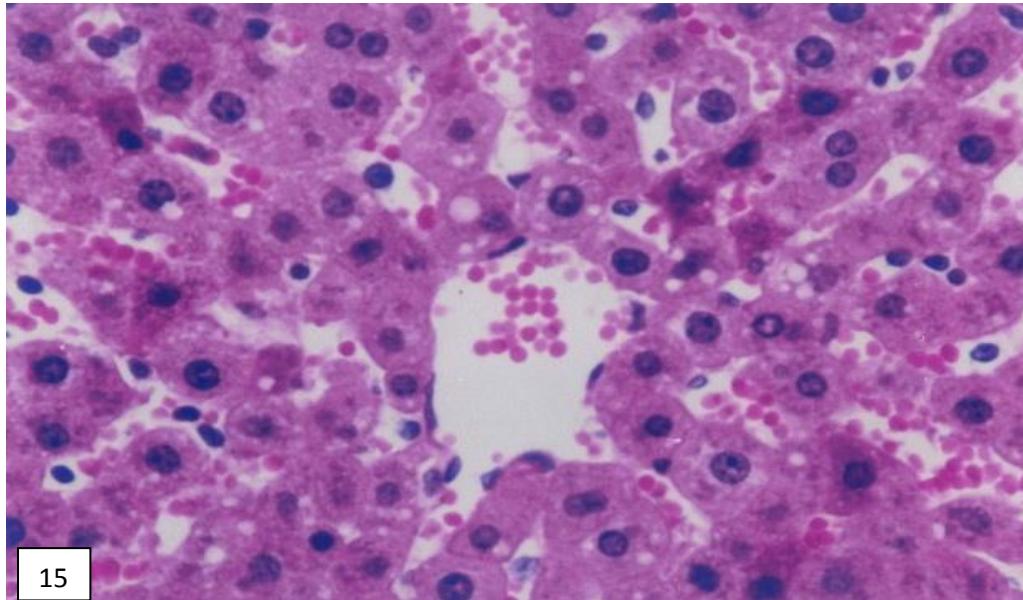


Fig (15): liver of rat treated by cord (1/8 LD50) showing dilated central vein and sinusoids (H&E X160).

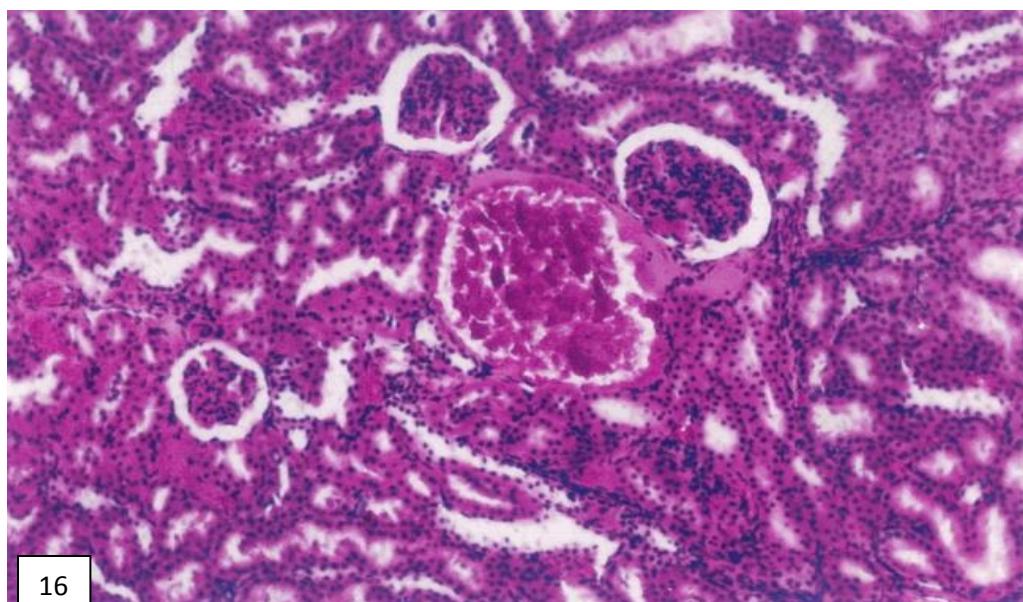


Fig (16): kidney of rat treated by cord (1/8 LD50) showing dilated cortical blood vessels (H&E X40).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

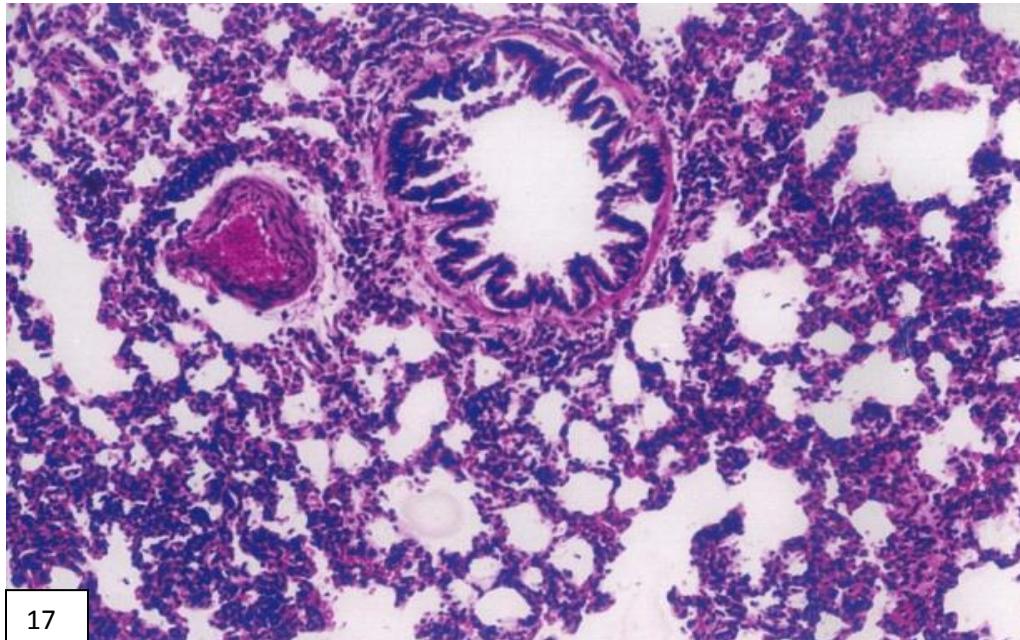


Fig (17): lung of rat treated by cord (1/8 LD50) showing dilated Peribronchiolar blood vessels and perialveolar capillaries (H&E X40).

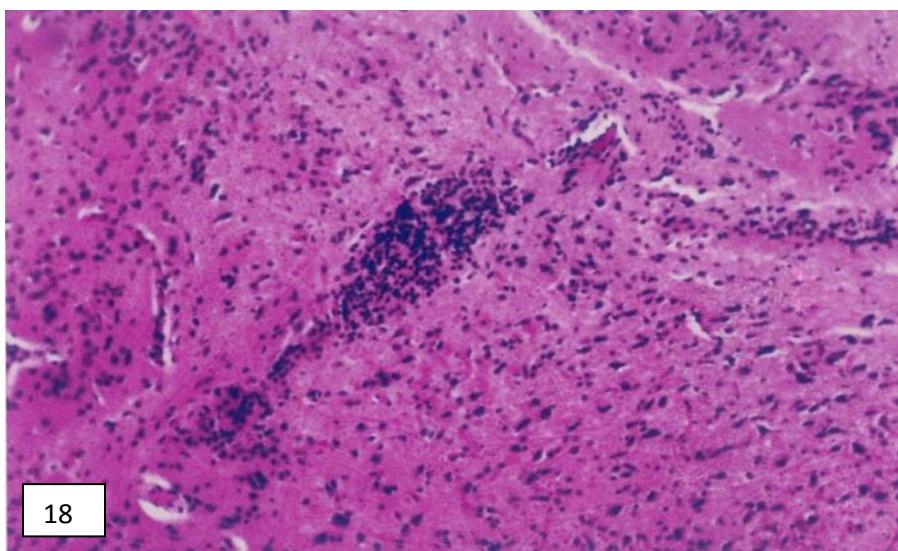


Fig (18): Brain of rat treated by cord (1/8 LD50) showing focal cerebral gliosis (H&E X40).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



DISSCUSION

Animals in the environments expose to ingest, inhale and absorb many chemicals that can impose stress on their organisms and trigger tissue damage by numerous biochemical mechanisms.

Our data revealed a high significant reduction of acetyl cholinesterase activity as result of administration of both tested compounds of Profenofos, specially high dose Ps-treated group. Such results are in agreement with **Goel et al.(2000)** and **Kamijima et al.(2004)**. Acetyl cholinesterase is the primary target of Organophosphorus insecticide. It is considered as a biochemical marker of exposure for Organophosphorus (**Rhman, et al., 2004**). **Rashed and Darwish (2002)** suggested that inhibition of acetyl cholinesterase in Organophosphorus poisoning occurred by formation of phosphorylated complex which are not easily hydrolyzed. Clinically sedation and paralysis were noticeable signs in our study due to inhibition of AchE activity in the experimental rats (**O'Brien, 1967**). Histopathological changes confirm the previous signs of intoxication. Where, brain showed focal gliosis and hemorrhage in the subarachnoid meningeal tissue of the cerebellum in high dose Ps-treated rats. While, focal gliosis in cerebral tissue were noticed in rats treated by Pc at the low and high dose. Lung revealed Peribronchiolar lymphoid hyperplasia associated with collapse and emphysema of the air alveoli in lung of high dose Ps-treated rats. Peribronchiolar and perialveolar blood capillaries were dilated and engorged with blood in low dose Pc-treated group.

The liver is the main organ responsible for detoxifying any foreign compounds entering the body. So, it uniquely exposed to a wide variety of exogenous and endogenous products ,these include environmental toxins and chemicals present in food and drinking water and juices (**Wight, 1982**). Since the liver is a primary site of biotransformation of foreign compounds, it is particularly vulnerable.

The obtained results indicated that an administration of rats with Ps at the dose of 46.30 mg/kg (1/4 of median lethal doses of selecrone) induced highly significant increase of AST and ALT ($P \leq 0.001$), while cord induced highly significant increase in AST and ($P \leq 0.001$) and significant rise in ALT ($P \leq 0.05$). 1/8 of median lethal dose of cord (22.31 mg/kg) proved high significant increase of AST.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



(Goel et al., 2000) reported that the increment of transaminases as the effect of Profenofos. The augmented levels of aminotransferases may be indicative of internal organs damage especially in liver and myocardium (Kaneko, 1997), it also could occur due to increased permeability and subsequent leakage of cellular enzymes (Varshneya et al., 1986). Hepatotoxic action of insecticide due to tissue damage seems to be indicative for elevation of liver enzymes in blood (Rahman et al., 2004). Elevation in the transaminases indicates the utilization of amino acids for the oxidation or for glucogenesis (Philip et al., 1995) and is used to determine liver damage.

Metabolism and biosynthesis of energetic macromolecules for different essential functions (Tordjor and Van Heen Star-Lequin, 1980). Shalby (2006) reported that one-tenth of LD50 of Profenofos induced significant increase in AST at 60 days of treatment (100.3% above normal level in rats) and ALT activity have the same trend of activity in case of AST.

Histopathological results confirm these data, in Ps treated group hepatic tissue revealed focal necrosis and proliferation of Kupffer cells as well as periductal inflammatory infiltration cells with fibroblastic proliferation surrounding the bile duct, association with sever dilation of central and portal veins and sinusoids, while Pc-treated group showed granular degeneration in the hepatocytes and Kupffer cells proliferation, in addition to focal mononuclear leucocytic aggregation.

Experimental toxicity after administration of some insecticide and pesticide may lead to destruction of the lipoprotein complex of the mitochondria in the cytoplasm of the hepatocytes with appearance of granular and fatty degeneration. Farrage and Shalby (2007) examined the liver sections of rats received to 1/10 of LD50, Profenofos and showed perportal necrosis of the hepatocytes near the portal area, dilated and congested portal vessels and mild area of inflammatory cell infiltration especially in the vicinity of the portal veins and near the bile ductules. Some cells exhibited necrosis together with Pyknosis of some nuclei. Slight hemorrhage was also noticed. EL-kashury et al.(2006) recorded dilation in the central veins and sinusoids with Kupffer cells proliferation appear in liver tissue Hammam and Abd El Mottaleb (2007) treated rates for 28 days with three different doses of Profenofos (1/20 LD50, 1/40 LD50, and 1/80 LD50). Liver showed hepatic cell damage with degenerative changes.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Farrag and Shalby (2007) studied the histochemical effects of Selecronine and examined liver sections stained with periodic acid Schiff's (PAS). The authors stated that a repeated oral administration of 1/10 LD₅₀ doses of Selecronine for two months showed a severe depletion in the polysaccharides content of the hepatocytes.

In current study, the high dose of cord induced significant increase in total protein due to the highly significant increase of albumin, while Selecronine proved significant rise of albumin only. The results are partially in parallel with that of **EL-kashury et al.(2006)**. They recorded that no changes in plasma total protein concentration was induced by Profenofos in rats, concerning with mean values of albumin it was noticed that a significant hyper albuminemia was noticed in low and high doses treated rats, this concur with similar investigation on Profenofos and other Organophosphorus which done by **Zidan et al. (1998)**. Hyper albuminemia may occurred as a compensatory mechanism when the need arises as in case of massive protein degradation to urea, liver increase albumin synthesis (**Gornall and Goldberg, 1980**).

Shalby (2006) recorded a gradual increase in the level of albumin in 1/10 LD₅₀ of Selecronine treated rats which reached its maximum after 60 days (90.8% above normal), but treated rats didn't return to normal level by the end of recovery period (+39.2%). Treated rats by 1/20 LD₅₀ of Selecronine caused significant increase in albumin concentration after 15 and 30 days of treatment (+12.5 and +17.8%), followed by slight reduction with the lapse of time (-21.7% after 30 days for recovery).

In the present work, high dose Ps-treated rats revealed significant increase in blood urea. Low dose of cord induced significant rise of creatinine level.

Shalby (2006) recorded that 1/10 LD₅₀ of Profenofos revealed significant increase in blood urea content, reached its peak at 60 days of treatment (83.6%), followed by gradual decrease without return to normal level (+29.1% after 30 days for recovery).

Mohideen and Raddy (1987) concluded that the activity of proteases which are responsible for various stages of protein degradation cascade in different tissues increased in Organophosphorus poisoning.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Increasing urea values can be explained by increasing tissue protein destruction as a signs of Profenofos toxicity resulting in high levels of urea which is the end product of protein catabolism in rat **EL-kashury et al. (2006)**. Similar observation in urea and creatinine concentrations were obtained in hepatorenal toxicity of Profenofos on rabbit and goat by **Ayyat et al. (2000) and Fakhr et al., (2000)** consequently. They also mentioned that Profenofos residue levels were found to be highest in liver and kidney tissues. **Kaneko (1997)** attributed these changes to toxic effect of insecticide on kidney causing some renal damage which resulted in a reduction of urea and creatinine excretion through urine. This reflected by histopathological examination; which represents some tissue alteration in kidney. Selecron-treated rats (high dose) showed hyperemia with focal hemorrhagic areas in the cortical area and corticomedullary junction. Uremia may be due to the decreasing role of Selecron on glomeular filtration, which subsequently raised the level of urea.

In cord-treated rats (low dose), renal tissue showed hyperemia in the cortical blood vessels. **Farrag and Shalby (2007)** reported that kidney of rat given oral doses equivalent to 1/10 LD50 of Profenofos day after day for two months exhibited inflammatory infiltration in the interstitial space. The renal corpuscles showed congestion and hyper cellularity and wide urinary space. The hemorrhage areas in the interstitium and the glomeruli were noticed. Some tubules show desquamation of its epithelial cells. Hammam and Abd El Mottaleb (2007) recorded hemorrhages, edema, necrosis and glomeruli shrinkage in kidney of rats treated for 28 days with Profenofos.

From these aforementioned results, it was concluded that two commercial products of Profenofos (Ps and Pc) caused alteration in biochemical parameters and tissue profile. The effects of (Ps) was more harmful than (Pc) on liver, kidney, lung and brain.

The adverse health effects are clearly minimized by selecting the right pesticide at proper time of application and using the right method.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



CONCLUSION

The comparative studies reveal that the two commercial products of Profenofos Ps and Profenofos Pc act as acetylcholinesterase (AchE) inhibitor. Moreover, induced some changes in level of certain biochemical constituents of blood, as well as, pathological alterations when given as sublethal doses into adult male albino rats for subacute and Subchronic time intervals. However, these alterations were more pronounced with Ps compound. Therefore, when we deal with pesticides, we have to pay appropriate attention to human health and environment conservation.

ACKNOWLEDGEMENT

The authors thank the central Agricultural Pesticides laboratory, Agricultural Research center- Cairo-Egypt. Deep appreciation to Prof. Dr. Afaf A. El-kashoury (Department of mammalian and Aquatic Toxicology) for her help and support.

REFERENCES

- Ayyat, M. S.; Abd El-Monem, U.M.; El-Gendy, H. M. and El-Fateh, H. M. (2000):** Profenofos effects on rabbit performance and their amelioration by using natural clay minerals. World Rabbit Science, 8(4):169-175.
- Bancroft, J. D. and Gamble, M. (2002):** Theory and practice of histological techniques 5th Ed. Curchil Livingstone, London Edinburgh, New York, Philadelphia, St. Louis, Sydney.
- Dumas, B. T.; Watson, W. A. and Biggs, H. G. (1971):** Quantitative colorimetric determination of albumin in serum or plasma. Clin. Chem. Acta, 31:87.
- El-Kashoury, A. A. and El-Far, F.A. (2004):** Effect of two products of Profenofos on thyroid gland, lipid profile and plasma APO-1/FAS in adult male albino rats, Egypt. J. Basic and Appl. Physiol., 3:213-226.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



El-Kashoury, A. F. A.; Amany, Y. Rashed and Kamal El- Said(2006): Some biochemical and pathologic alterations in male albino rats exposed to Profenofos insecticide. Egypt. J. Basic Appl. Physiol, 5(1): 135-148.

El-Kashoury, A. A. (2009): Influence of subchronic exposure of Profenofos on biochemical markers and microelements in testicular tissue of rats. Nature and Science, 7(2):16-31.

Ellman, G. L.; Dlane, K.; Courtney,V.;Andres, J.R. and Deatherstone, R. M. (1961):A new and rapid colorimetric determination of acetyl cholinesterase activity. Biochem. Pharmacol.,7:88-95.

Fakhr, I. M. I.; Fouzy, A. S. M. and Hazzaa, N. I.(2000): Effect of C14 Ethoxy Profenofos insecticides on liver and kidney functions of lactating goats. Egypt. J. Vet. Sci.,34:49-58.

Farrag, H. Abdel Razik and Shalby, E. M. Shehata (2007): Comparative histopathological and histochemical studies on IGR, Lufenuron and Profenofos insecticide albino rats. J.Appl.Sci. Res., 3(5):377-386.

Fawcett, J.K. and Scott, J.E. (1960): Determination of urea (Urease modified Berthelot reaction). J. Clin. Pathol., 13:156-159.

Goel, A.; Chanhan, D. P. and Dhawan, D. K. (2000): Protective effect of zinc in chlorypyrifos induced Hepatotoxic : a biochemical and trace element study. Biol. Trac. Elel. Res., 74(2):171-183.

Gornall, A. G. and Goldberg, D. M. (1980): Hepatobiliary disorders. In: Applied Biochemistry of clinical Disorders. Atlan G. Gornall, Inc. Virginia Venue, Hegerstown, Maryland.

Greisha, S.; Saad, M.; Mosleha, Y.; Loutfy, N.; Dessoukib, A. A. and Ahmeda, M. T. (2011): Human Risk Assessment of Profenofos:A case study in Ismailia, Egypt. Polycyclic Aromatic compounds,31(1):28-47.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Hammam, F. M. and Abd el Mottaleb, E. M. (2007): Studies of the Genotoxic and Histopathological effects of the Organophosphorus insecticide "Profenofos" on white rats. The Egypt. J. Hospi. Med., 29:658-706.

Hanafy, M. S. M.; Arbid, M. S. and Afify, M. M. H. (1991): Biochemical and histopathological effects of the Organophosphorus insecticide (Tamaron) in rats. Indian. J. Anim. Sci., 61:43-47.

Kamijima, M.; Hibi, H.; Gotoh, M.; Taki, k. and Saito, I.; Wang, H.; Itohara, S.; Yamada, T.; Ichihara, G.; Shibata, E.; Nakasima, T. and Takeuchi, y.(2004): A survey of semen indiees in insecticide sprayers. J. Occup. Health, 46: 109-118.

Kaneko, J. J. (1997): Clinical Biochemistry of domestic animals.5th Ed.-Academic Press Inc. Harcourt Brace-Jovanovich, Berketey, Boston, London, Sydney, Tokyo, Toronto.

Leng, G.; Kuhn, K. H. and Idel, H. (1997a): Biological monitoring of pyrethroids in blood and pyrethroid metabolism in urine: application and limitations. Sci. Total Environ. 199:173-181.

Leng, G.; Leng, A.; Kuhn K. H.; Lewalter, J. and Pauluhn, J. (1997b): Human dose-excretion studies with the pyrethroid insecticide cyfluthrin urinary metabolite profile following inhalation xenobiotica, 27:1272-1283.

Mikhail, T.H.; Aggour, N.; Awadallah, R.; Boutos, M.N.; El-Dessoukey, E.A. and Karima, A.I.,(1979): Acute toxicity of Organophosphorus and organochlorine insecticides in laboratory animals. Z. Ernahrungswiss., 18:258-268.

Mohideen, M. B. and Raddy, R. M. (1987): Changes in the brain profiles of fresh water fish Cyprinus caripo under malathion stress. Zeitschrift. Fur Angewandte, Zoologic, 74(3):293-297.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



O'Brien, R. D. (1967): Insecticide Action and metabolism. Academic Press, New York, 55:88.

Oluah, N. S. (1998): Effect of sublethal copper (11) ions on the serum transaminase activity in catfish Clarias albopunctatus. J. Aquat. Sci., 13:45-47.

Philip, G. H.; Reddy, P. M. and Sridevi, G. (1995): Cypermethrin induced in vivo alterations in the carbohydrate metabolism of freshwater Wsh Labeo rohita, Ecotoxicol. Environ. Saf., 31:173-178.

Rahman, M. F.; Mahboob, M. F. and Grover, P.(2004): Comparative sensitives of in vitro acetyl cholinesterase inhibition by novel Organophosphorus compounds in broiler chicken. Toxicology international . 11(1):49-53.

Rashed, A. Y. and Darwish, F. M. M. (2002): Protective effect of casein and/or Ascorbic acid on some metabolic and Patholgical changes induced by Malathion insecticide in rats. Vet. Med. J. Giza, 50(40).

Reitman, S. and Frankel, S. (1957): A colorimetric method of the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminses. Am. J. Clin. Path.,28:57-63.

Schalm, O. W. (1986): Veterinary Haematology. 4th Ed. Lea and Febiger, Philadelphia, pp.21-86.

Shalby, E. M. Shehata (2006): Comparative hematological and hepatorenal toxicity of IGR, Lufenuron and Profenofos insecticide on albino rats. J. Egypt. Soc. Toxicol. 34:85-98 Jan.

Siest, G.; Henny, J.; Schiele, F. and Young, D.S.(1958): Kinetic determination of creatinine. Interpretation of Clincl Laboratory Test (1985),pp.220-234.

Snedecor,G. V. and Cochran, W. G. (1967): Statistical Methods. 6th Ed., Iowa State Univ. Press. Ames, Iowa, USA.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Tordior, W.F. and Van Heem, E. A. Stra-Lequin (1980): Field studies monitoring exposure and effects in the development of pesticides. Elsevier, Amsterdam., Oxford, New York :207.

Varshneya, C.; Behgan, H. S. and Sharma, L. D. (1986): Effect of dietary malathion on hepatic microsomal drug metabolizing systems of Gallus domesticus. Toxical. Letter, 31:107-111.

Weichselbaum, T.E. (1946): Quantitative colorimetric determination of total protein in serum. Am. J. Clin. Path., 7:40.

Weil,C.S. (1952): Tables for convenient calculation of medium effective dose (LD50 or ED50) and instruction in use. Biometrics, 8:249-263.

Wight, D. G. D. (1982): Atlas of liver pathology. Lancaster, MTP Press Ltd.

Zidan, Z. H.; Mashhour, A. K.; Zidan, A. A.; Fawzy, A. A. and Okasha, Y.A.(1998): Toxicological effect of long term administration of minimal doses of certain insecticides on white albino rats. 7th conf. Agric. Dev. Res., Annals Agric. Sci., Ain Shams Univ., Cairo, Egypt, 15-17 Dec.,198, sp Issue3, 1085-1101.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



EXPERIMENTAL INDUCTION OF HYPERTHYROIDISM IN FEMALE BALADI GOATS WITH SPECIAL REFERENCE TO SOME BIOCHEMICAL AND PATHOLOGICAL PARAMETERS

A.M. Bakeer*, Iman B. Shaheed*, Sherein, S.A El Gayed*, Reda M.S. Korany* and Naela M. Ragaa**

*Pathology Department, Faculty of Veterinary Medicine, Cairo University, Egypt.

**Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Cairo University, Egypt.

ABSTRACT

This study was conducted to investigate the effect of experimental induction of hyperthyroidism on ten female Baladi goats, five months old. Levothyroxine sodium at the rate 1300 µg/animal/day as oral drench was used for four weeks to induce hyperthyroidism. Hormonal assay showed significant increase in both T₃ and T₄ levels, whereas total lipid showed significant decrease in its level. Clinically the animals showed round areas of alopecia at the face and ear with emaciation. Gross examination of the sacrificed goats revealed enlargement of kidneys and liver with distention in the gall bladder. Histopathologically, the kidneys revealed vacuolar degeneration in the epithelium of renal tubule and endothelium of glomerular tuft. Liver of goats with induced hyperthyroidism showed vacuolar degeneration and necrosis of hepatocytes. Microscopic examination of the thyroid glands of affected cases revealed large dilated follicles with abundant colloid, the lining epithelium of the follicles were low cuboidal to squamous (colloid goiter).

Keywords: hyperthyroidism, goat, kidneys, liver, thyroid, goiter

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



INTRODUCTION

The thyroid hormones, tetraiodothyronine or thyroxine (T4) and triiodothyronine (T3) act on many different target tissues, stimulating oxygen utilization and heat production in every cell of the body. The overall effects are increasing the basal metabolic rate (**Todini et al., 2006**)

Breed, age, sex and season are the most frequent factors that affect the concentration of thyroid hormones (**Tuckova et al., 2001**). The changes of blood thyroid hormone concentrations are an indirect measure of the changes in thyroid gland activity and circulating thyroid hormones and can be considered as indicators of the metabolic and nutritional status of the animals (**Todini, 2007**).

Aim of work: This study was planned to study the thyrotoxic effect of levothyroxine sodium on the thyroid glands and some other organs of goats as an experimental animal model, and also its effect on thyroid hormonal assay {triiodothyronine (T3) and thyroxin (T4)}.

MATERIALS AND METHODS

I-Animals:-

Ten Baladi female goats, five months of age, 10- 12 Kg body weight were used. They were purchased from El-Fayoum governorate Markets. Goats were acclimatized for seven days before the onset of the study, they were fed balanced ration and hay. The drinking water was offered ad libitum.

II-Experimental design:

The goats were randomly divided into two groups. The 1st group (contain four goats) served as a control. The 2nd group (contain six goats) was given levothyroxine sodium (Euthyrox® tablet) which were obtained from Amoun Company at the rate of 1300 µg/animal/day as oral drench for four weeks.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



The clinical signs and the changes in behavior were recorded. Two goats from the 1st group and three goats from the 2nd groups were slaughtered at the end of each two weeks of the experiment.

III-Postmortem and histopathological examination:

At the time of slaughter the goats were subjected to postmortem examination after slaughtering to detect any abnormal gross changes. Tissue specimens from kidneys, liver, thyroid glands, skin of goats were collected, fixed in 10 % neutral buffered formalin, processed and embedded in Paraffin wax, sectioned at 4 μm and stained with Hematoxylin and Eosin and Prussian blue (**Bancroft and Gamble, 2008**)

IV-Clinicopathological examination:

The whole blood was collected from goats at the time of slaughter in plain centrifuge tubes. The blood was centrifuged at 3000 rpm/ 5 minute for serum separation and then kept in sterile test tubes at -20°C till used for determination of total T3 and total T4 using RADIOIMMUNOASSAY (RIA) kits (Beckman Coulter, Immunotech a.s., Czech Republic) according to the method described by Nixon et al., 1988. The bound radioactivity was determined in a gamma counter set for ^{125}I iodine. A standard curve was constructed and unknown values were obtained from the curve by interpolation. Assay sensitivity was 0.3 and 13 nmol/l for T3 and T4 respectively. Intra and inter-assay coefficients of variation (CV) were respectively 6.3% and 7.7% for T3 and 6.2% and 8.6% for T4 assay.

Total lipid was by enzymatic colorimetric method (kits obtained from Bio Analytics, Palm City, FL) according to method of **Stein, 1986**.

V-Statistical analysis:-

The obtained data from experimental animals were statistically analyzed by SPSS 14 version for Windows. The differences between groups were determined with variance analysis (one-way

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



analysis of variance [ANOVA]). When the differences were significant, Student-Newman-Kuels test was performed. All data were recorded on an individual basis. Data were expressed as means \pm standard error (SE).

RESULTS

Concerning to group 1 (Control group), There was no any abnormal clinical signs or pathological changes were detected in this group.

Group 2 (goats treated with levothyroxin sodium), showed round areas of alopecia especially at the face and ear in 2nd week of the experiment. The same lesion was observed at the 4th week of the experiment (Fig. 1). The goats of this group showed poor body condition and emaciation.

After two weeks from the onset of the experiment, gross examination of the animals treated with levothyroxine sodium revealed; large pale kidneys, while microscopical finding showed slight hydropic degeneration of lining epithelium of renal tubule associated with vacuolation of lining endothelium of glomelular tuft (Fig.2).

Concerning to liver, gross finding revealed distended gall bladder. Microscopical findings were irregular vacuoles inside the cytoplasm of hepatocytes with irregular outline cells contour associated with periductal mononuclear (lymphocytes, macrophages and plasma cells) inflammatory cells infiltration with fibroplasia (Fig. 3). Individual cell necrobiosis in hepatocytes was detected (Fig. 4).

There was no any macroscopic alteration detected in thyroid glands of this group, while microscopical examination revealed large dilated follicles with abundant colloid, while the lining epithelium of the follicles showed low cuboidal to squamous epithelium (Fig. 5). The previous mentioned description was categorized under (colloid goiter).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Gross finding of skin showed round areas of alopecia especially at the face and ear of the experimental animals. The histopathological examination of skin of treated goats revealed signs of alopecia represented by complete absence of hair follicles. Some areas showed atrophied hair follicles.

Animals treated with levothyroxine sodium at the fourth week from the start of the experiment revealed mild paleness of the kidneys. Microscopic examination showed degeneration of lining epithelium of renal tubule and vacuolization in the lining endothelium of glomelular tuft (Figs. 6&7).

Concerning the liver, gross examination revealed distended gall bladder, while microscopical findings were degenerative changes of hepatocytes with multiple irregular vacuoles in the cytoplasm, while the nuclei were centrally located. There were portal as well as periductal fibrosis with hyperplasia of bile duct epithelium (Figs. 8). There were congestion of hepatic blood vessels and activation of Kupffer cells in all examined cases (Fig.9).

There was no observable gross abnormality in thyroid gland, while microscopic examination revealed cystically dilated follicles with large amount of colloid. The lining epithelium of the follicles showed low cuboidal to squamous lining epithelium (Fig. 10).

There was round areas of alopecia especially at face and ear. The previous lesions which were recorded in the skin at 2nd week of the experiment were detected also at the 4th week. (Figs. 11&12).

Hormonal assay of this group showed significant increase in both T3 and T4 levels, whereas total lipid showed significant decrease in its level (Table 1 &2).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

**Table (1)** : Thyroid hormonal assay and total lipid of goats after two weeks from the experiment

Group	T3(nmol/L)	T4(nmol/L)	Total lipid(mg/dl)
Control	3.05±0.577 ^b	97.18±0.577 ^b	502.38±0.577 ^b
Hyperthyroidism	6.37±0.94 ^a	190.56±55.61 ^a	431.73±0.408 ^c
P value	0.001	0.05	0.0001

Table (2) : Thyroid hormonal assay and total lipid of goats after four weeks from the experiment

Group	T3(nmol/L)	T4(nmol/L)	Total lipid(mg/dl)
Control	5.66±0.577 ^b	95.08±1.154 ^b	502.38±0.577 ^b
Hyperthyroidism	6.11±0.408 ^a	316.50±47.458 ^a	453.57±7.56 ^c
P value	0.0001	0.001	0.0001

The values are the mean ± standard error of mean (SEM)

Values with different super script are significantly different at $p \leq 0.05$

2013

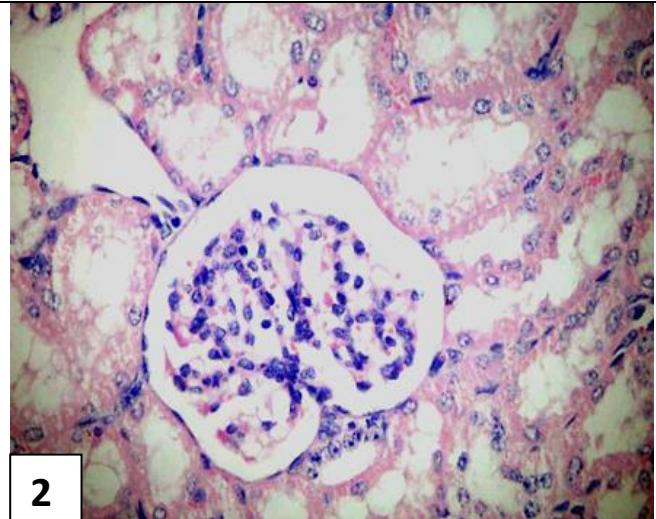
Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

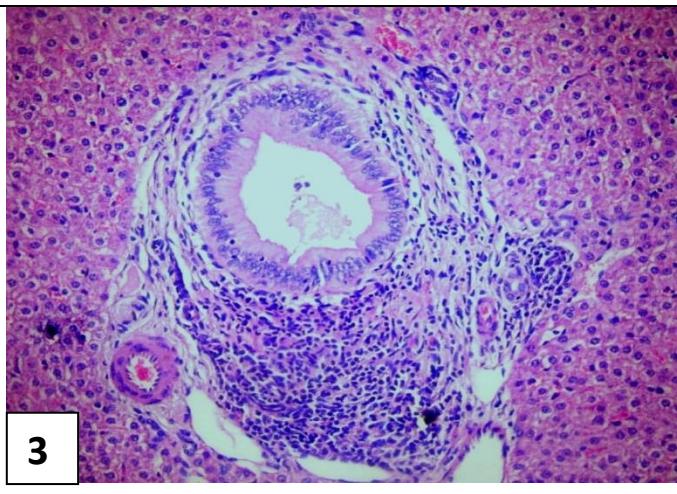
April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



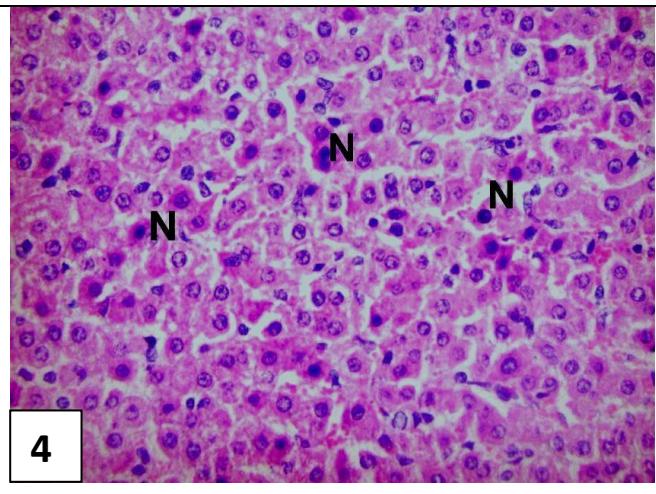
1



2



3



4

Fig. 1: Goat treated with levothyroxine sodium after four weeks from the experiment showing round area of alopecia at face and ear.

Fig. 2: Micrograph of Kidney, goat treated with levothyroxine sodium after two weeks. Note slight vacuolar degeneration of lining epithelium of tubule (V) and vacuolation of endothelium of glomerular tuft. (H&E X 40)

Fig. 3: Micrograph of Liver, goat treated with levothyroxine sodium after two weeks. Note periductal mononuclear inflammatory cells infiltration at portal area with fibroplasia. (H&E X 20)

Fig. 4: Micrograph of Liver, goat treated with levothyroxine sodium after two weeks, showing individual cells necrosis (N). (H&E X 40)

2013

PATHOLOGY CONFERENCE

Faculty Of Vet. Med. - Cairo Univ.



April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

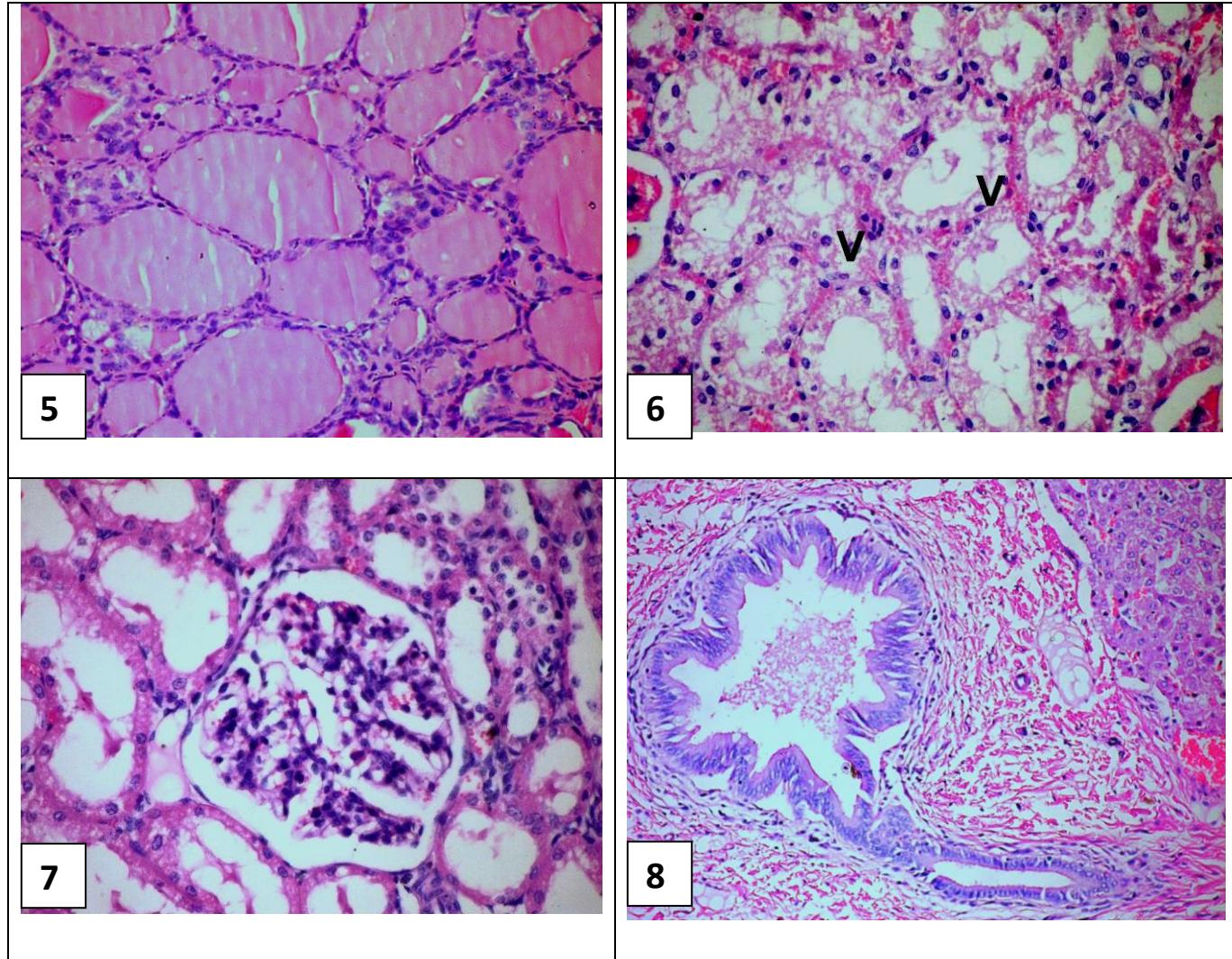


Fig. 5: Micrograph of thyroid, goat treated with levothyroxine sodium after two weeks. Notice inactive thyroid follicles that filled with large amount of colloid material which pressed on the epithelial lining. (H&E X 40)

Fig. 6: Micrograph of Kidney, goat treated with levothyroxine sodium after four weeks, showing vacuolar degeneration and necrosis of tubular epithelium (V). (H&E X 40)

Fig. 7: Micrograph of Kidney, goat treated with levothyroxine sodium after four weeks. Note vacuolation of glomerular lining endothelium. (H&E X 40)

Fig. 8: Micrograph of Liver, goat treated with levothyroxine sodium after four weeks. Notice periductal fibrosis and hyperplasia of the lining epithelium of the duct. (H&E X 20)

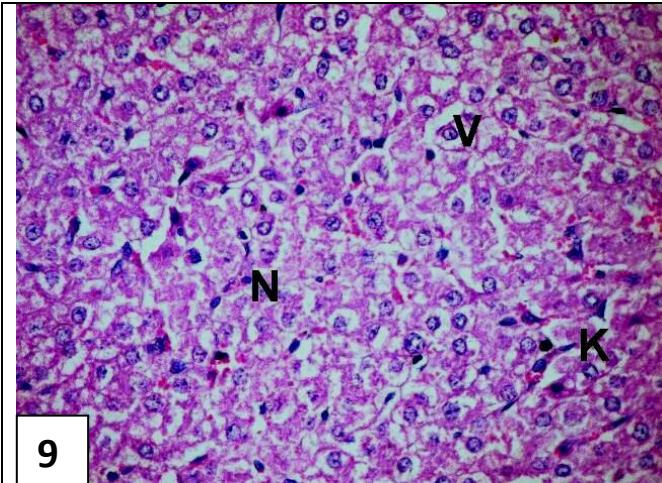
2013

PATHOLOGY CONFERENCE

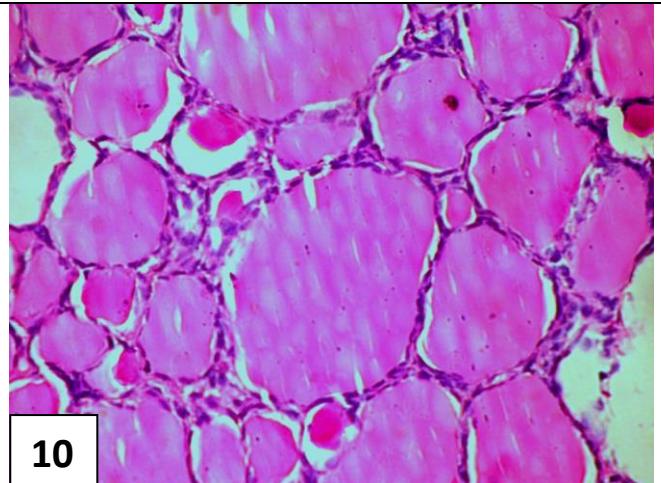
Faculty Of Vet. Med. - Cairo Univ.



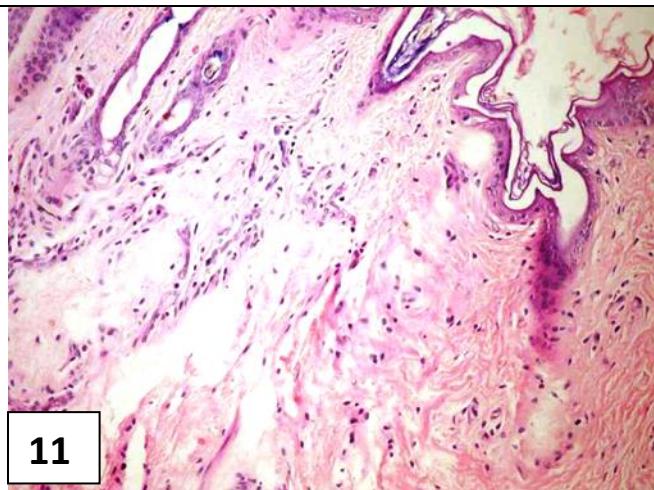
April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



9



10



11



12

Fig.9: Micrograph of Liver, goat treated with levothyroxine sodium after four weeks. Note vacuolar degeneration (V) and necrosis (N) in hepatocytes with proliferation of Kupffer cells (K). (H&E X 40)

Fig. 10: Micrograph of Thyroid, goat treated with levothyroxine sodium after four weeks, showing cystic dilatation of follicles that filled with colloid and lined by low cuboidal to flattened epithelium. (H&E X 40)

Fig. 11: Micrograph of Skin, goat treated with levothyroxine sodium after four weeks, showing area devoid of hair follicle. (H&E X 20)

Fig. 12: Micrograph of Skin, goat treated with levothyroxine sodium after four weeks, showing atrophied hair follicles. (H&E X 20)

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



DISCUSSION

The experimental study was designated to investigate the effect of hyperthyroidism on some body organs and hormonal assay especially T3 and T4 and also total lipids in goats.

Hyperthyroidism induced significant increase in T3 and T4 and this result was compatible with that recorded by **Panciera et al., 1989**. Total lipid decreased significantly and this result was observed by **Varas et al., 2001**.

Half of circulating T3 is derived from deiodination of T4 in peripheral tissue, so peripheral conversion of T4 is the principal source of T3 in animals administered thyroxin (**Panciera et al., 1989**) and this could be interpret our result in increasing the level of T3 in case of thyroxin treatment.

Hyperthyroidism affects lipid metabolism in liver and induces hypolipemia (**Varas et al., 2001**).

The clinical signs appeared on goats of this group were, round areas of alopecia at their face and ear and this sign was not mentioned in the available references, with poor body condition and emaciation and these result was mentioned by **Vegad and Katiyar, 2000**. These signs may be due to direct thyrotoxicosis which cause dermatosis and inturn skin alopecia, and increased basal metabolic rate and increased body catabolism which inturn cause emaciation and poor body condition.

Few studies discussed the pathological changes in different organs in case of hyperthyroidism.

Liver of hyperthyroid animals showed vacuolar degeneration and necrosis of hepatocytes and this picture was similar to that observed by **Lawrence et al., 1991**.

Degenerative changes in the liver in animal suffered from hyperthyroidism were believed to be evidence of direct toxic effect of thyroxin on hepatocytes (thyrotoxicosis) (**Lawrence et al., 1991**).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Microscopic examination of kidneys revealed presence of vacuolar degeneration of lining epithelium of renal tubule and lining endothelium of glomelular tuft. This change may be also as a result of thyrotoxicosis as in liver as mentioned by **Lawrence et al., 1991**.

Results of microscopic examination of the thyroid glands of experimental animals of this group revealed large dilated follicles with abundant colloid, the lining epithelium of the follicles were low cuboidal to squamous (colloid goiter) and this result was previously recorded by Panciera et al., 1990. Alterations of the pituitary thyroid axis were reflected in the histologic appearance of the thyroid glands. The thyroid glands reflected decreased TSH secretion with decreased epithelial hight and increased colloid volume. It is known that thyroid glands become atrophied when the trophic effects of TSH are lacking. The flattened epithelium and increased colloid were due to TSH deficiency secondary to thyroxine administration (**Panciera et al., 1990**)

From these results we concluded that,

- 1- Experimental hyperthyroidism has moderate effects on different body organs (kidneys, liver, thyroid glands and skin) and also some blood parameters (T3,T4 and total lipid).
- 2- Hyperthyroidism induced colloid goiter.

REFERENCES

Bancroft, J.D. and Gamble M., (2008):- Theory and Practice of Histopathological Techniques.

6th edition, Churchill Livingstone. New York, London and Madrid.

Lawrence, D.; Thompsom, J.; Layton, A.W.; Calderwood-Mays, M.; Ellison, G. and mannella, C., (1991):- Hyperthyroidism Associated With a Thyroid Adenoma in a Dog. JAVMA, 1991; 199(1): 81-83

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Nixon, D.A.; Akasha, M.A.; and Anderson, R.R., (1988):- Free and total thyroid hormones in serum of Holstein cows.J. Dairy Sci., 1988; 71:1152-1160

Panciera, D.L.; Atkins, C.E.; Bosu, W.T.K. and MacEwen, E.G., (1990):- Quantitative Morphological Study of the Pituitary and Thyroid Glands of Dogs Administered L-thyroxine.Am. J. Vet. Res., 1990; 51(1):27-31

Panciera, D.L.; MacEwen, E.G., Atkins, C.E.; Bosu, W.T.K.; Refsal, k. R. and Nachreiner, R.F., (1989):- Thyroid Function Tests in Euthyroid Dogs Treated with L- thyroxine.Am. J. Vet. Res., 1989; 51(1):22-26

Stein, E.A., (1986):- Quantitave enzymatic colorimetric test for determination of plasma total lipid and cholesterol .Text Book of Clinical Chemistry, pp.879-886, W.B.Saunders, Philadelphia.

Todini, I., (2007):- Thyroid hormones in small ruminant: effect of endogenous, environmental and nutritional factors. Animal, 1(7): 997-1008

Todini, I.; Delgadillo, J.A., Debenedetti, A. and Chemineau, P., (2006):- Plasma total T3 and T4 concentrations in bucks as affected by photoperiod. Small ruminant research, 2006; 65: 8-13

Tuckova, M.; Kozak, M.; Fialkovicova, M.; Palenik, L.; Magic, D. J. and Bekeova, E., (2001):- Age and the concentration of thyroid hormones in dogs .Folia Veterinaria. 2001; 45(4, Supplementum): 59-62

Varas , S.M.; Jahn , G.A and Gimenez, M.S., (2001):- Hyperthyroidism affects lipid metabolism in lactating and suckling rat. Lipids, 2001; 36 (8):801-6

Vegad, J.L. and Katiyar , A.K.(2000):- A Textbook of Veterinary Systemic Pathology. Vikas Publishing House PVT. LTD. Delhi.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



CLINICAL AND PATHOLOGICAL EVALUATION OF FOUR DIFFERENT SUPERFICIAL NEOPLASMS IN DOGS

Reda M.S. Korany* and Khaled M. Abd elhakim**

*Pathology Department, Faculty of Veterinary Medicine, Cairo University, Egypt.

**Police Officer, Police Academy, M.V.Sc., Faculty of Veterinary Medicine, Cairo University, Egypt.

ABSTRACT

In this study, four different types of neoplasms were reported in dogs. Clinical, macroscopic and histopathological examinations were performed for all cases. First case was five years old male German Shepherd, which had an overgrowth about 3 cm in diameter at its jaw. Histopathological examination revealed parotid adenoma. This dog was treated by surgical excision of the mass with no recurrence. Second case was three years old male German Shepherd, and had an overgrowth about 7 cm in diameter at its perineum. Via microscopic examination, this mass was diagnosed as lipoma. Cure occurred by surgical excision. Third case was three years old female Great Dane, with a multiple, ulcerated and easily bled overgrowths around external genitalia which were highly invasive. Microscopically; the tumor was diagnosed as mastocytoma. Euthanasia was performed due to extensive invasion. Fourth case was male German Shepherd, four years old with single, irregular mass about 2 cm in diameter at its back. Microscopically, it was diagnosed as squamous cell carcinoma. The case treated by surgical excision with no recurrence.

Keywords: dogs, neoplasms, adenoma, lipoma, mastocytoma, squamous cell carcinoma.

1st International Scientific conference of Pathology Department, Faculty of Veterinary Medicine (191-203).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



INTRODUCTION

Neoplasms in dogs are twice more frequent in comparison to man, and progress more rapidly and bear similar anatomical and physiological properties, proving them as an excellent animal model for understanding human cancers (**Hahn et al., 1994**). Skin tumors are relatively frequent, especially in dog, horses, bovines and cats (**Lakatos et al., 2009**)

In dogs, skin and subcutis are the most common sites for neoplasms, accounting about 67.5% of all neoplasms (**Duncan and Prasse, 1979**)

Lipoma, adenoma, and mast cell tumor are the most common skin tumors in dogs (**Villamil et al., 2011**).

Salivary gland tumors are rare in dogs and mandibular salivary gland is the most frequently affected followed by parotid one (**Smrkovski et al., 2006**)

Lipomas occur approximately in about 16% of dogs. They are benign tumors of fat cells, most common in adult female or elderly obese dogs. Lipomas usually occur as solitary masses, but multiple lipomas can also occur in dogs. These tumors are frequently localized in the subcutaneous tissue but may extend intramuscularly or along deep facial planes (**Lamagna et al., 2012**).

Mast cell tumors (MCTs) are one of the most common tumors of the canine skin, estimated to represent up to 20 percent of all skin tumors in this species and have a reputation for being difficult to manage because of their variable clinical presentation, behavior and response to treatment (**Dobson and Scase, 2007**), In the UK, MCT is the second most common canine malignancy, after soft tissue sarcoma with an incidence of 129 per 100,000 insured dogs per year (**Dobson et al., 2002**).

Squamous cell carcinoma (SCC) is relatively more common neoplasm of all neoplasms that are known to affect dogs (**Chandrashekaraiah et al., 2011**).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Aim of work: this report was conducted to identify some of skin tumors which are common neoplasm in canine species.

MATERIALS AND METHODS

Four dogs with superficial overgrowth were examined both clinically and histopathologically. These cases were obtained from K-9 department clinic at police academy and private clinic in Cairo, Egypt.

Data of each case was recorded including breed, age, sex, case history, and clinical findings.

Tumors were excised under general anesthesia using atropine sulphate (0.05mg per kg. S.C.) and xylazine Hcl (0.1mg per kg. I.M.) as preanesthetics, ketamine Hcl (10mg per kg. I.M.) and thiopental sodium (25mg per kg. 2.5% I.V.) as maintenance.

Tissue specimens from each case were collected, fixed in 10 % neutral buffered formalin, processed and embedded in Paraffin, sectioned at 4 μm , then stained with Hematoxylin & Eosin (H&E) and Toluidine blue (**Bancroft and Gamble, 2008**) and examined using light microscope (Olympus, Japan).

RESULTS

In this study four types of neoplasms were diagnosed on the basis of the gross and histopathological examination.

First case was tumor obtained from male German Shepherd, five years old, which had an overgrowth about 3 cm in diameter at its jaw (Fig 1). The mass grossly was circumscribed, encapsulated, firm, lobulated, white and glistening in cut section (Fig 2).

Histopathologically, this mass was formed of acini of variable sizes and shapes and its lumen contains serous fluid; it lined by cuboidal epithelium, its cytoplasm contains numerous

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



basophilic granules; nuclei are uniform, round and in basal half of cell with intact basement membrane. Cyst and cyst-papillary adenoma was detected in some areas. Connective tissue stroma contained well-developed blood vessels was observed between the glands (Fig 3&4). This mass was diagnosed as **parotid adenoma**. Dog treated by surgical excision with no recurrence.

Second case was male German Shepherd, three years old and had an overgrowth about 7 cm in diameter at its perineum (Fig.5). The clinical examination of this overgrowth revealed soft, multiple, white or even congested lobulated masses of variable shapes and sizes.

Microscopically, it was characterized by large, adipose cells with flattened and compressed nuclei (Signet ring) (Fig.6). Each group of cells were separated by scanty fibrous connective tissue stroma with presence of prominent capsule; blood vessels in some areas were congested. This mass was diagnosed as **lipoma**. Treatment was occurred by surgical excision.

Third case was female Great Dane, three years old with a multiple, ulcerated and easily bled overgrowths surrounding external genitalia and they were highly invasive (Fig.7), Microscopically, the tumor was composed of groups of mast cells (large cell with deeply esinophilic granules and large eccentric basophilic nuclei) separated by hyalinized connective tissue stroma (Fig.8). By Toluidine blue stain the cytoplasm of mast cells was filled with metachromatic granules (deep violet) (Fig.9). Tumor was diagnosed as **malignant mastocytoma**. Euthanasia was performed due to marked infiltration of tumor into surrounding tissue.

Fourth tumor was obtained from male German Shepherd, four years old with single, irregular overgrowth about 2 cm in diameter at its back (Fig.10). Gross examination revealed irregular, white, fleshy mass (Fig.11). Microscopically it was characterized by infiltration of dermal layer by tumor cells which arranged into small nests and showed mitotic activities, with prominent central lamellated keratin pearl (Fig.12). This mass was diagnosed as squamous cell carcinoma. The case treated by surgical excision with no recurrence.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



In conclusion, neoplasms are common in dogs, and their types resemble human ones. Four naturally occurring types of tumors were described in this study.

DISCUSSION

Cutaneous neoplasms hold a central position in the skin pathology of the dog (**Georgeta et al., 2011**).

Lipoma, adenoma, and mast cell tumor are the most common skin tumors in dogs (**Villamil et al., 2011**).

In this study we described four different types of neoplasms occurred in two different breeds of dogs.

First type was parotid adenoma. The mass grossly was circumscribed, encapsulated and firm, histopathologically it was formed of acini of variable sizes and shapes; it lined by cuboidal epithelium with intact basement membrane. This result was resemble to that recorded by **Shimoyama et al., 2006**.

Canine salivary gland tumors are rare in dogs and broadly classified into benign and malignant. The submandibular gland is the most frequently affected followed by parotid one (**Smrkovski et al., 2006**).

The differentiation between benign and malignant salivary tumor is very difficult, however this tumor was well circumscribed, showed no criteria of malignancy with no atypical mitosis and no pleomorphism, suggesting a benign salivary adenoma (**Ozaki and Narama, 2003**).

Second recorded one was lipoma, which characterized by large, adipose cells with flattened and compressed nuclei and it is similar to that explained by **Lamagna et al., 2012**.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Lipomas are common benign tumours of fat cells. In most cases, surgical excision is curative and easy to be performed, Lipomas usually occur as solitary masses, but multiple lipomas can also occur in dogs, these tumors are frequently localised in the subcutaneous tissues but may extend intramuscularly or along deep fascial planes (**Lamagna et al., 2012**). Benign cutaneous neoplasms of adipose tissue occur in all species and simple lipomas are common in dogs (**Liggett et al., 2002**).

As humans, lipomas are common in the dog; however, until now there were no reports of cytogenetic investigations on these tumors in the canine (**Reimann et al., 1999**).

Third type was mastocytoma, and composed of groups of mast cells (large cell with deeply esinophilic granules and large eccentric basophilic nuclei) separated by hyalinized connective tissue stroma and this result was in agreement with that mentioned by **Simoes et al., 1994**. By Toluidine blue stain, the cytoplasm of mast cells was filled with metachromatic granules (deep violet).

Mast cell tumors are among the most common skin neoplasms in dog. They tend to occur in middle-aged to elderly dog. Mast cell tumors in dogs are potentially malignant (**Simoes et al., 1994**). Mast cell tumor, cutaneous histiocytoma, squamous cell carcinoma, fibrosarcoma and melanocytoma were the most common tumors, accounting over 50% of all the skin tumors (**Moraes et al., 2009**). These tumors are potentially malignant neoplastic processes belonging to the group of round cell tumors, which correspond to 7–21% of the skin neoplasms affecting dogs, with no sexual predisposition (**Trefeuzzi et al., 2003**). If mast cells were in large numbers, mast cell tumor could be diagnosed by cytology alone. In preparations containing few cells, mast cell tumor may be difficult to differentiate from inflammatory processes containing mast cells (**Duncan and Prasse, 1979**).

Fourth case was male German Shepherd, four years old with single, irregular overgrowth about 2 cm in diameter at its back. Gross examination revealed irregular, white, fleshy mass,

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



similar observation has been reported by **Chandrashekaraiah et al., 2011**. Microscopically it was squamous cell carcinoma and characterized by infiltration of dermal layer by tumor cells which arranged into small nests and showed mitotic activities, with prominent central lamellated keratin pearl and this result was in agreement with **Moraes et al., 2009**.

Squamous cell carcinoma (SCC) is relatively more common neoplasm of all neoplasms that are known to affect dogs. It occurs in dogs in age ranging from 2 to 14 years (**Chandrashekaraiah et al., 2011**) Available literature revealed that skin, nasal planum, oral cavity, tongue and digits were the most common sites where SCC was reported (**Viswanath et al., 1998**). Explanation for the high occurrence of squamous cell carcinoma in the previous studies may relate to the long periods for which dogs remain in environments exposed to high rates of solar radiation (**Moraes et al., 2009**). SCC shows varying features from incomplete carcinoma in intra epidermal form to highly malignant tumor type in its invasive form (**Burkhard et al., 2001**).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

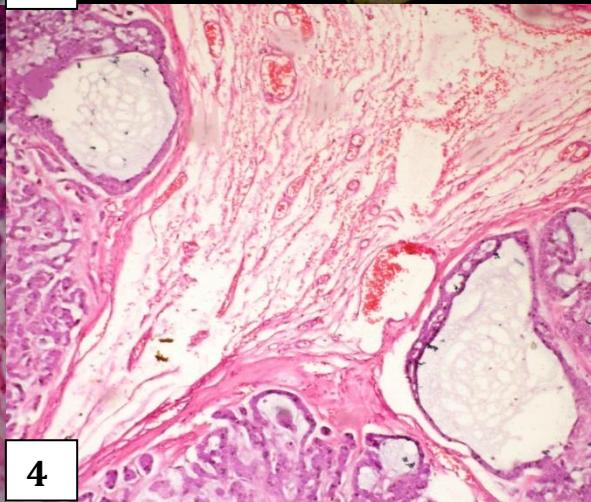
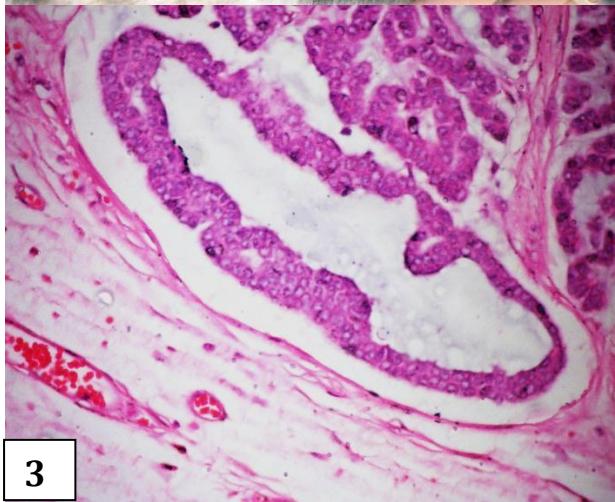
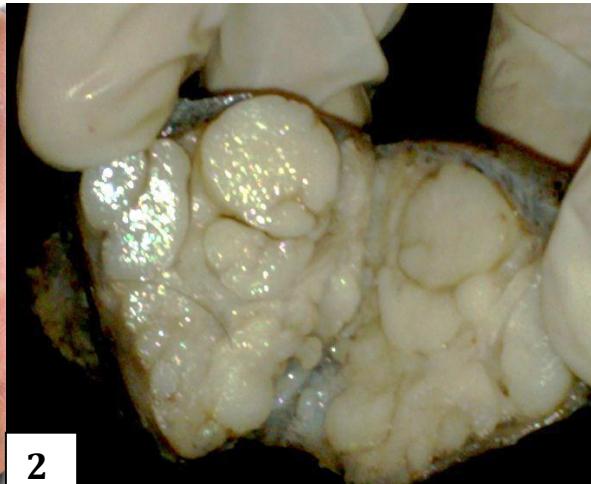
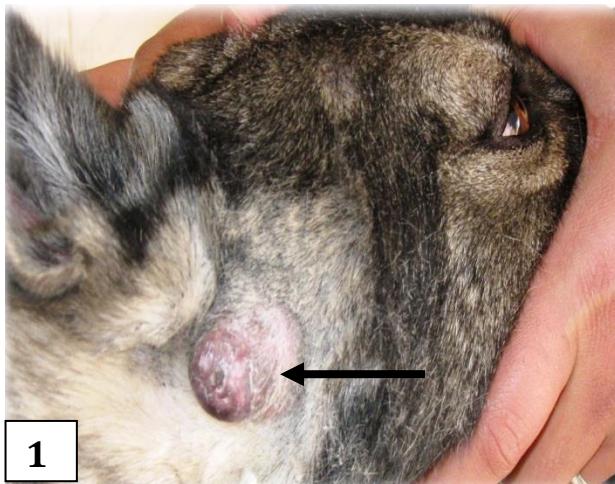


Fig. 1: Parotid adenoma, male German Shepherd, five years old, note circumscribed, encapsulated overgrowth at jaw (arrow).

Fig. 2: Cut section of the previous overgrowth. Notice lobulated, white and glistening tumor mass.

Fig. 3: Micrograph of parotid adenoma showing acini of variable sizes and shapes; it lined by cuboidal epithelium, cyst-papillary adenoma was detected. Connective tissue stroma contained well-developed blood vessels between the glands (H&E X 200).

Fig. 4: Micrograph of parotid adenoma. Note cyst adenoma with vascularized connective tissue stroma (H&E X 200).

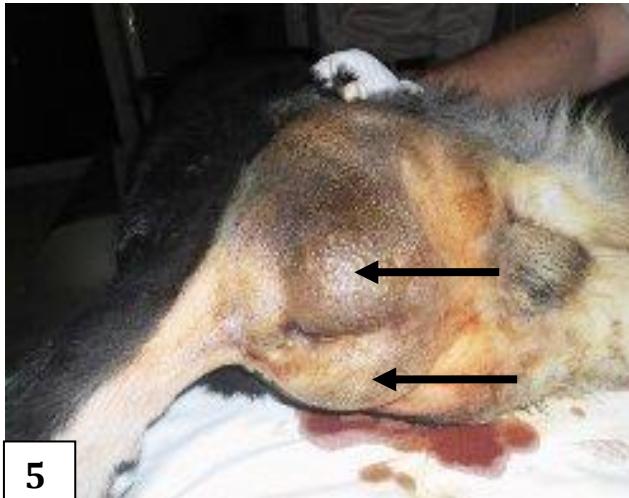
2013

PATHOLOGY CONFERENCE

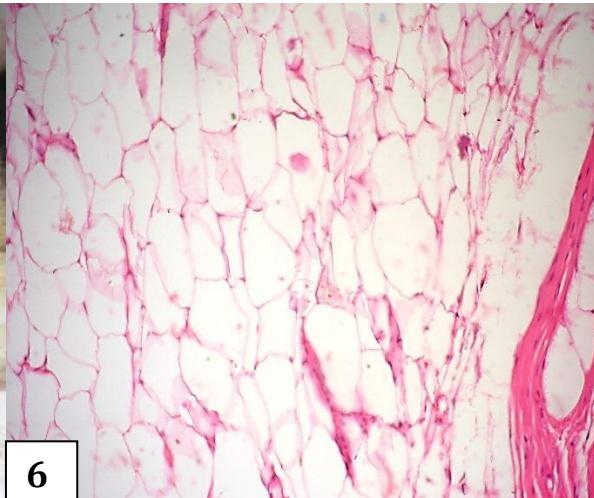
Faculty Of Vet. Med. - Cairo Univ.



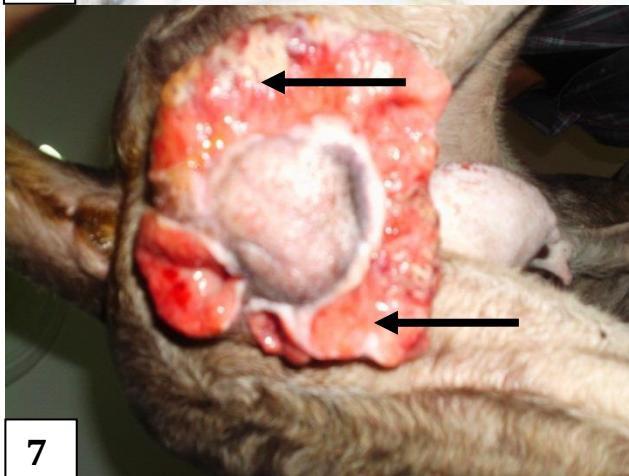
April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



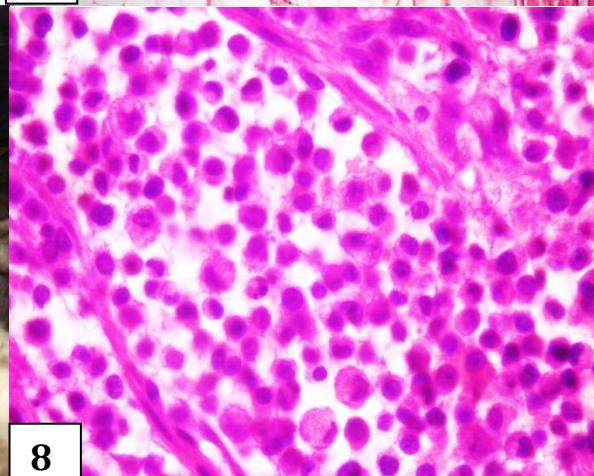
5



6



7



8

Fig. 5: Lipoma, male German Shepherd, three years old showing multiple overgrowth at perineal region (arrows).

Fig. 6: Micrograph of lipoma. Note large, adipose cells with flattened and compressed nuclei (H&E X 400).

Fig. 7: Mastocytoma, female Great Dane, three years old with a multiple, ulcerated overgrowths surrounding external genitalia (arrows).

Fig. 8: Micrograph of mastocytoma showing groups of mast cells separated by connective tissue stroma (H&E X 1000).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center

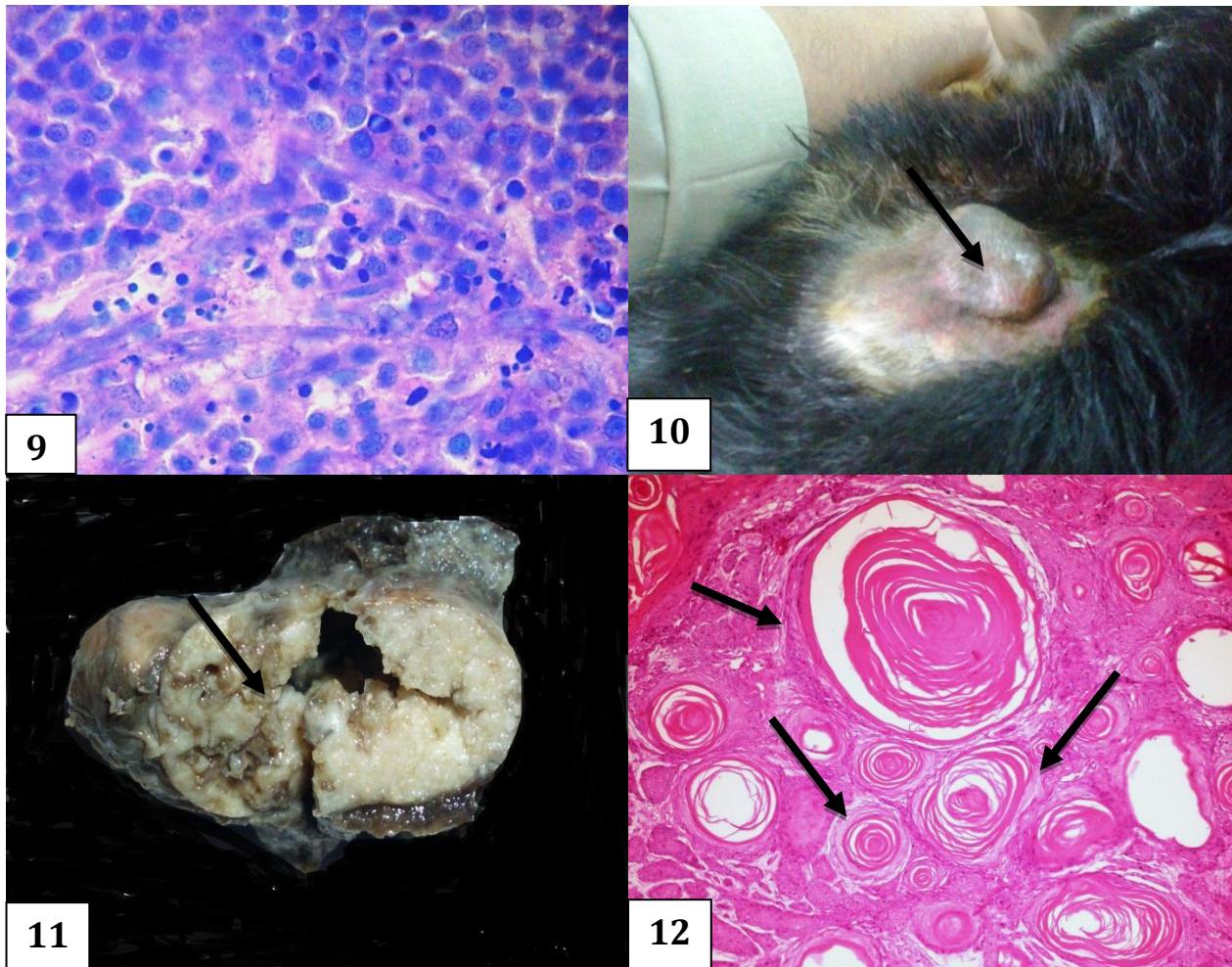


Fig. 9: Micrograph of mastocytoma by Toluidine blue stain, mast cells metachromatic granules appeared deep violet (X 1000).

Fig. 10: Squamous cell carcinoma, male German Shepherd, four years old with single, irregular overgrowth at its back (arrow).

Fig. 11: Cut section of the previous overgrowth showing white and fleshy tumor mass (arrow).

Fig. 12: Squamous cell carcinoma. Note infiltration of dermal layer by tumor cells which arranged into small nests with prominent central lamellated keratin pearl (arrows) (H&E X 100).

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



REFERENCES

- Bancroft, J.D. and Gamble M., (2008):** Theory and Practice of Histopathological Techniques. 6th edition, Churchill Livingstone. New York, London and Madrid.
- Burkhard MJ.; Valenciano A. and Barger A., (2001):** Respiratory tract. In: Atlas of Canine and Feline Cytology. 1st Ed., W.B. Saunders, Philadelphia, pp135-185.
- Chandrashekaraiah, G. B.; Rao, S.; Munivenkatappa, B.S. and Mathur, K. Y., (2011);** Canine Squamous Cell Carcinoma: a Review of 17 Cases. *Braz. J. Vet. Pathol.*, 4(2): 79-86
- Dobson, J. M., Samuel, S., Milstein, H., Rogers, K. & Wood, J. L. N. (2002):** Canine neoplasia in the UK: estimates of incidence rates from a population of insured dogs. *Journal of Small Animal Practice*, 43: 240-246
- Dobson, J. M. and Scase, T. J., (2007):** Advances in the diagnosis and management of cutaneous mast cell tumours in dogs. *Journal of Small Animal Practice*, 48(8): 424-431
- Duncan, J. R. and Prasse, K. W. (1979):** Cytology of Canine Cutaneous Round Cell Tumors: Mast Cell Tumor, Histiocytoma, Lymphosarcoma and Transmissible Venereal Tumor. *Vet. Pathol.*, 16: 673-679
- Georgeta, D.; Iancu, F.; Condrut, E.; Curtseit, S.; Ciobotaru, E. and Militaru, M., (2011):** Overview of the Cutaneous Benign Epithelial Tumors in Dog - Epidemiological and Morphological Aspects. *Scientific Works, C Series*, vii (1): 225-233
- Hahn, K.A.; Bravo, L.; Adams, W.H. and Frazier, D.L. (1994):** Naturally occurring tumors in dogs as comparative models for cancer therapy research. *In Vivo.*, 8:133-43
- Lakatos, I.; Mirela E.; Cedar, A.L. and Baba I. (2009):** Cutaneous Tumors' Incidence In Dog. *Lucrari stiinlifice medicina veterinara*, Xlii (2): 375-381
- Lamagna B, Greco A, Guardascione A, Navas L, and Ragozzino M, (2012):** Canine Lipomas Treated with Steroid Injections: Clinical Findings. *PLoS ONE* 7(11): e50234. doi:10.1371

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Liggett, A. D.; Frazier, K. S. and Styer, E. L., (2002): Angiolipomatous Tumors in Dogs and a Cat. Vet. Pathol., 39 (2): 286-289

Moraes, J. R. E., Beretta, D. C. S., Zanetti, A. S., Garrido, E., Miyazato, L. G., Sevarolli, A. L. (2009): Skin Tumors in Dogs- a Retrospective Study of Ten Years. Veterinária Notícias, 15 (1) : 59-68

Ozaki, K. and Narama, I, (2003): Pleomorphic Adenoma of The Salivary Gland in Two Prairie Dogs. J. Toxicol. Pathol., 16: 171-173

Reimann, N.; Nolte, I.; Bonk, U.; Bartnitzke, S. and Bullerdiek, J., (1999): Cytogenetic Investigation of Canine Lipomas. Cancer Genetics and Cytogenetics, 111(2): 172-174.

Shimoyama, Y.; Yamashita, K.; Ohmashi, T.; Akihara, Y.; Sako, T.; Hirayama, K.; Okamoto, M. and Taniyama, H, (2006): Pleomorphic Adenoma of the Salivary Gland in Two Dogs. J. of Comp. Pathol., 134(2-3): 254-259.

Simoes, J. P. C.; Schoning, P. and Butine, M. (1994): Prognosis of Canine Mast Cell Tumors: A Comparison of Three Methods. Vet. Pathol., 31: 637

Smrkovski, O. A.; LeBlanc, A. K. ; Smith, S. H; LeBlanc, C. J.; Adams W. H. and Tobias K. M., (2006): Carcinoma ex Pleomorphic Adenoma with Sebaceous Differentiation in the Mandibular Salivary Gland of a Dog. Vet. Pathol. 43:374–377

Trefezzi, R.S.; Xavier, J.G.AND Catao-Dias, J. L., (2003): Morphometry of Canine Cutaneous Mast Cell Tumors. Vet. Pathol. 40:268–275

Villamil, J. A; Henry, J; Bryan, N; Ellersieck, M; Schultz, L; Tyler,W and Hahn, W, (2011): Identification of the most common cutaneous neoplasms in dogs and evaluation of breed and age distributions for selected neoplasms. Journal of the American Veterinary Medical Association, 239 (7):960-965.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Viswanath S.; Vijayasarathi S.K.; Gowda R.N. and RAO, S., (1998): Pathology of canine oral neoplasms. Indian J. Vet. Pathol., 22: 150-153.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Speaker Biographies

Mary M. Christopher, DVM, PhD, Dipl ACVP, Dipl ECVCP, Professor, Department of Pathology, Microbiology, and Immunology , School of Veterinary Medicine , University of California-Davis, USA

Dr. Mary Christopher is a Professor of Clinical Pathology at the University of California-Davis School of Veterinary Medicine. She is board-certified by the American College of Veterinary Pathologists and the European College of Veterinary Clinical Pathology. Dr. Christopher obtained her DVM from Iowa State University and her PhD at the University of Minnesota. At UC Davis, she served as Chief of Clinical Pathology, Associate Director of the Teaching Hospital, and Assistant Dean of Academic Programs. She was Editor-in-Chief of *Veterinary Clinical Pathology* from 1997-2009, founded the International Association of Veterinary Editors, and teaches scientific writing. Dr. Christopher has authored more than 100 peer-reviewed research publications in hematopathology, cytology, and clinical chemistry, and given many invited presentations around the world. She was a 2010-11 Fulbright Scholar at Alexandria University in Egypt. She was awarded the 2011 Hall of Fame Award from the European Society for Veterinary Clinical Pathology and the 2010 Education Award from the American Society for Veterinary Clinical Pathology. She is the Executive Director of the International Society for Animal Clinical Pathology.

Prof. Dr. Mahmoud Attia (BVMSC, DVP, Ph.D.)

More than 48 years of experience in toxicology and toxicological pathology. He is Expert pathologist and independent consultant at Attia consultancy, Evreux, France since 2008. From **1986-2008** he was Scientific Director, Director of histopathology and clinical pathology and Expert pathologist at CIT (Center International of Toxicology), Evreux, France. From **1975-1986** he was Leading pathologist/toxicologist /head pathology and clinical pathology in drug safety

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



R&D labs, at NV Organon, Oss, The Netherlands. From **1972-1975** he was Senior lecturer of Pathology at Faculty of Veterinary Medicine Cairo University, Egypt. From **1968-1972** he was research scientist (Ph.D student) in Pathology/Clinical Pathology at Moscow Veterinary Academy, Moscow, Russian Federation. From **1966-1968** he was lecturer assistant of pathology/clinical pathology at the Faculty of Veterinary medicine, Cairo university, Egypt.

Dr. Mahir Abdul Ghani Kubba (BVMS, MSc, Ph.D)

He is associated professor in College Of Veterinary Medicine, Tripoli University. He graduated and took his master degree from College of Veterinary Medicine, Baghdad University, Iraq. He got his Ph. D. from Glasgow University, Glasgow, United Kingdom.

Marco Patruno Vincenzo (Ph.D)

He is Associate Professor of Veterinary Anatomy at the Department of Comparative Biomedicine and Food Science, University of Padova since 2011. He got degree with full marks in Animal Science in 1995, at University of Milan, Faculty of Veterinary Medicine. He was student work placement in University of London, Division of Physiology Thomas's Hospital at 1/1994 to 11/1995. He got his Ph.D. degree in Embriology at Royal Holloway, University of London, UK at 2001. He is author of more than 100 international publications of which 49 full papers, 9 chapters in books of international interest and 66 abstracts.

He invited as speaker at Royal Swedish Academy of Sciences, Kristineberg Marine Research Station (2002), at the Society for Experimental Biology meeting (2003) held in Southampton (UK), at the Stem Cell Symposium (2010) held in Tropea (Italy), at the Tissue Engineering Conference (2012) held in Chicago (USA) and at the 1st Scientific Workshop on Stem Cells for Ligament and Tendon Tissue Engineering and Regeneration (2013) held in Pescara, Italy. He was reviewer of projects for national and international agencies (ISF, ALW, Italian MIUR, and others) as well as of scientific papers for a number of international journals.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



**Prof. Dr. Yesari Eroksuz (Ph.D). Firat University, School of Veterinary Medicine,
Pathology Department, 23 119, Elazig-Turkey**

Dr. Yesari Eroksuz graduated from Veterinary School in the class of 1982 at the University of Istanbul, Turkey. After two years of military service, he began a pathology doctorate programme at the University of Firat in 1989.

After completing his doctorate training in 1996, Dr Eroksuz stayed at the University of Firat as a faculty member in anatomic pathology. He first became interested in toxic pathology subjects, specifically pyrrolizidine alkaloidosis in animals, anatomic pathology of ruminants and ocular pathology of small animals. He visited Tuft, Wisconsin and Hokkaido Veterinary Schools of pathology departments for 3 months as a guest scientist. He was the charter faculty member of the Pathobiological Sciences where he remains as Professor of Pathology.

Dr. Kamal Hossein Abdel Tawab Zidan (MVSc)

He got a Bachelor of Veterinary Medicine, Faculty of Veterinary Medicine, Cairo University, Egypt at 1997. He got at 2002 diploma of Microbiology Faculty of Veterinary Medicine, Cairo University. Also he got his Master's Degree in Veterinary Microbiology at 2004. Nowday he prepares for his Ph.D in Veterinary Microbiology. He work in many poultry company for about 10 years, accompanied with working in private farms of large and small animals. He Work in Ministry of agriculture, Egypt, General authority for veterinary services as well as Work now in Ministry of agriculture, KSA, Al-Riyadh Veterinary Diagnostic laboratory.

Prof. Dr. Hatice Eroksuz (Ph.D)

Firat University, School of Veterinary Medicine, Pathology Department, 23 119, Elazig-Turkey

Dr. Hatice Eroksuz graduated from Veterinary School at the University of Elazig, Turkey in 1987. She began a pathology doctorate programme at the same university in 1988.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



After completing his doctorate training in 1993, Dr Eroksuz stayed at the University of Firat as a faculty member in anatomic pathology. She became interested in toxic pathology subjects, and especially anatomic pathology of avian species and the use of immunohistochemical techniques. She visited Tuft and Hokkaido Veterinary Schools of pathology departments for 3 months as a guest scientist. She was the charter faculty member of the Pathobiological Sciences where she remains as Professor of Pathology and chairman since 2009.

Dr. Sherein Saied Abdelgayed (Ph.D)

Assistant Professor of Pathology Faculty of Veterinary Medicine, Cairo University, Egypt.

Sherein Saied Abdelgayed was born in Cairo, Egypt, in 1971. She received the B. Sc. degree in veterinary medicine from Cairo University, Cairo, Egypt, in 1994. She joined the Department of Pathology, Faculty of veterinary medicine, Cairo University as a Demonstrator in 1995. In 1999, she got the M. Sc. degree and became an assistant lecturer of Pathology till 2004 when she received the Ph.D. degree and became a lecturer of Pathology. She is currently an Associate Professor of Pathology , Pathology department, Faculty of Veterinary medicine, Cairo University, Egypt. Dr. Sherein was a member and group leader of the research team of the project entitled “ Establishment Of Economical Protocol For Continuous Production Of Fasciola Antigen Used For Vaccine And Diagnostic Kit Production “ 2004 – 2008. She is a Life Member of Egyptian Veterinary Medical Association and Egyptian Veterinary Medical Society of Pathology and Clinical pathology.

Dr. Mohamed Abdel Rhman Ibrahim Bosila. (MVSc)

Assistant Researcher of Poultry Diseases, Veterinary Researches Division, National Research Center, Egypt.

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



Mohamed graduated from faculty of Veterinary medicine, Cairo University in 1998. He got his master degree in pathology in 2007. He almost finished his Ph. D thesis in pathology of poultry and will discuss it soon.

Prof. Dr. El sayed Rashad Abdel-Meguid El- Attar

Professor of Pathology Faculty of Veterinary Medicine Zagazig University,Egypt

Dr. Sayed was graduated at 1980 from Faculty for veterinary medicine ,zagazig university. He got his master degree at 1985 and Ph.D at 1988. He got training course on parasitic immunopathology particularly immunohistochemistry and In-Situ Hyperdization from School of medicine,Tufts University ,Boston,U.S.A from 1991- 1992

Dr. Khadra Soliman (Ph.D)

Pathology Department, Animal Health Research Institute, Dokki, Giza.

She Graduated from Faculty of Veterinary Medicine, Cairo University at 1988. She got her master degree at 1996 and Ph.D at 2004 in Pathology from Faculty of Veterinary Medicine, Cairo University. She worked as researcher at Tissue Culture Unit, King Fahd Medical Research Center at 2003-2004. She worked as Associate Professor in Al-Riyada Collage for Health Sciences at period from 2006-2009. Now she work as pathologist in Pathology Department, Animal Health Research Institute, Dokki, Giza..

Dr. Mohamed Abdelrazik Salah.

Assistant lecture , Histology Department , Faculty of Veterinary Medicine, Cairo Univerisity, Giza, Egypt.

He graduted from Faculty of Veterinary medicine, Cairo university at 2006. He got his master degree at 2011. He work on his Ph. D thesis "Virual Microscopy for learning , assessment and

2013

Faculty Of Vet. Med. - Cairo Univ.

PATHOLOGY CONFERENCE

April 25-27, 2013 Sheraton Dreamland Hotel & Conference Center



application on some genital organs of She-Camel (*Camelus Dromedarius*) ". Also Dr. Mohamed has interest in Human resource management. He got Professional Human Resource Management Diploma from Cairo University at 2010 and Advanced HRM Diploma at 2011.