

ISSN: 2322-4568



An international peer-reviewed journal which publishes in electronic format

Volume 6, Issue 1, March 2016

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**Muhammad Abdullahi Mahmud**, DVM., M.Sc., SENIOR LECTURER, Department of Animal Health and Production Technology, Niger State College of Agriculture, Mokwa, Niger State, **NIGERIA**; Email: [drmahmud2@gmail.com](mailto:drmahmud2@gmail.com)

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**MUHAMMAD SAEED**, Ph.D (Student), Animal Nutrition and Feed Science, College of Animal Sciences and Feed technology, Northwest A&F University, Yangling, 712100, **CHINA**; Email: [muhammad.saeed@nwsuaf.edu.cn](mailto:muhammad.saeed@nwsuaf.edu.cn)

**Osman Erganiş**, PhD. Professor of Microbiology; Department of Microbiology, Faculty of Veterinary Medicine, Selcuk University, Konya, **TURKEY**; Email: [oerganis@selcuk.edu.tr](mailto:oerganis@selcuk.edu.tr)

**Shewangzaw Addisu Mekuria**, BSc., MSc., Instructor, department of Animal Production and Extension, University of Gondar, P. O. Box 196, Gondar, **ETHIOPIA**; Email: [shewangzaw.addisu@uog.edu.et](mailto:shewangzaw.addisu@uog.edu.et); [shewangzaw21@yahoo.com](mailto:shewangzaw21@yahoo.com)

**Terry Ansah**, PhD., Nutrition - Ruminants; University for Development Studies-Ghana and Harper Adams University College, **UNITED KINGDOM**; Email: [ansahterry@yahoo.com](mailto:ansahterry@yahoo.com)

**Thakur Krishna Shankar Rao**, PhD, Assistant professor, Vanabandhu College of Veterinary Science & Animal Husbandry, Navsari Agricultural University, Navsari Gujarat, **INDIA**; Email: [tkrao.vet@gmail.com](mailto:tkrao.vet@gmail.com)

**Vassilis Papatsiros**, Professor, Dietary input, Animal and Feed interactions; Porcine Medicine, Faculty of Veterinary Medicine, University of Thessaly, Trikalon str 224, GR 43100, **GREECE**; Email: [vpapatsiros@vet.uth.gr](mailto:vpapatsiros@vet.uth.gr)

**Wafaa Abd El-Ghany Abd El-Ghany**, PhD, Assistant Prof. of Poultry and Rabbit Diseases; Poultry and Rabbit Diseases Department, Faculty of Veterinary Medicine, Cairo University, Giza, **EGYPT**; Email: [wafaa.ghany@yahoo.com](mailto:wafaa.ghany@yahoo.com)

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Email: [wesleylyeverton@yahoo.com.br](mailto:wesleylyeverton@yahoo.com.br)

**Yagoob Garedaghi**, Assistant professor, PhD of Parasitology; Department of Veterinary Parasitology, Tabriz Branch, Islamic Azad University, Tabriz, **IRAN**; Email: [y\\_garedaghi@iaut.ac.ir](mailto:y_garedaghi@iaut.ac.ir)

**Sesotya Raka Pambuka**, M.Sc., Sinta Prima Feedmill, Poultry and Aqua Feed Formulation, Sulaiman Rd 27A, West Jakarta, **INDONESIA**; Email: [sesotya-raka@sintafeed.com](mailto:sesotya-raka@sintafeed.com)

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**Sina Vahdatpour**, DVM-DVMS, Faculty of Veterinary medicine, Tabriz Branch, Islamic Azad University, Tabriz, **IRAN**; Email: [sinavahdatpour@yahoo.com](mailto:sinavahdatpour@yahoo.com)

**Tohid Vahdatpour**, PhD, Assistant Prof., Physiology; Dep. Animal Sciences, Shabestar Branch, Islamic Azad University, Shabestar, **IRAN**; Email: [tvahdatpour@gmail.com](mailto:tvahdatpour@gmail.com)

Volume 6 (1); March 25, 2016 [XML]

Research Paper

**Effects of Drugs on Serum Biochemical Profile and Renal Failure in Humans and Experimental Rats: A Preliminary Report.**

Al shafei NK and Nour A

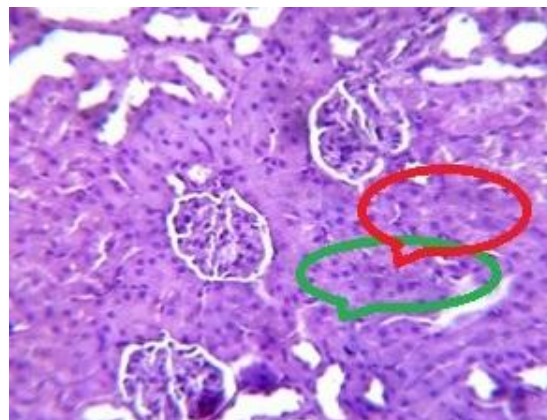
*World's Vet. J.* 6(1): 01-09, 2016;

pii:S232245681600001-6

**ABSTRACT**

Antibiotics and cancer treatment drugs have been associated with renal failure. In developing countries, antibiotics are often obtained without prescription and this misuse increases the risk of renal failure, especially for patients which have been taking gentamicin for a long-term. For example, taking the aminoglycoside antibiotic, particularly in diabetics, without close medical supervision has been suggested to the cause kidney damage and renal failure in many patients. The objectives of this preliminary study were to investigate the association of antibiotics and renal failure in Hemodialysis (HD) patients in Egypt; and to document the changes in biochemical profiles and histopathology of the kidneys when nephrotoxicity is induced by the antibiotic gentamicin (GM) and the cancer therapeutic agent Cisplatin (Cis). Serum biochemical profiles of HD patients and rats with induced renal failure were compared. Fifty HD patients and six normal people were employed in the study. Sixty experimental rats were divided into three groups: Control, gentamicin-induced renal failure group, and cisplatin-induced renal failure group. The survey of HD patients revealed that 6% of the renal failure is antibiotics related. GM and Cis caused kidney damage and renal failure in rats. The levels of blood serum urea, creatinine, and potassium significantly increased in drug-induced renal failure rats. Serum sodium decreased in GM rats with renal failure and in HD patients compared to the control groups. The histopathological changes in renal tissues in Cis and GM induced renal failure appeared in early stage of renal dysfunction. More studies are needed to determine the correlations between biochemical markers and histopathological changes that may be used as an early warning system for assessing earlier drugs-induced stress to the kidneys before renal failure progresses.

**Key words:** Gentamicin, Cisplatin, Drug-Induced renal failure, Hemodialysis, Serum biochemical profile



[Full text-[PDF](#)] [RiCeST]

Research Paper

**Evaluation of Using Honey, Cool Water and Levamisole against Heat Stress on Different Traits of Rabbits under Egyptian Summer Conditions.**

El Saïdy NR., Allam FE, Balabel TM and El-Midany SA.

*World's Vet. J.* 6(1): 10-18, 2016;

pii:S232245681600002-6

**ABSTRACT**

This study was conducted in order to estimate the impact of using honey in drinking water, drinking cool water and Levamisole injection as alleviated tools of heat stress on White New Zealand rabbits under Egyptian summer conditions. 40 sexually mature White New Zealand rabbits contained 36 does with an average age of 15-20 (18±2) weeks and nearly similar body weight of 2 kg and 4 fertile bucks with an average age of 24 weeks and average weight of 2.5 kg were used in this experiment. They were allocated into four groups each containing 9 does and 1 buck. Group I was kept as a control without any treatment, group II received honey 20 ml/l on drinking water, group III drank cold water with a temperature ranged from 16-20 °C and group IV received a single dose of subcutaneous Levamisole injection (2 mg/kg BW). Results showed that most of performance, reproductive and physiological parameters of examined rabbits were significantly decreased by heat stress under Egyptian summer conditions. However, treated groups showed improved traits on most examined parameters comparing with control group. Among the treated groups, the one treated with honey expressed significant increase (P<0.05) in body weight, body weight gain, feed intake, feed conversion ratio, water consumption, conception rate, litter size and weight, milk yield, economic efficiency, rectal temperature, hematological parameters and some serum biochemical parameters. On the other hand, the group which received cool water showed the best records for decreased levels of serum urea, creatine and respiratory rate. In conclusion, it is clear that heat stress has negative effects on reproductive and physiological traits of growing rabbits with drawing attention toward the importance of using alleviating methods for mitigating the negative effects of heat stress especially by using honey and drinking cool water.

**Key words:** Heat stress, Honey, Cool water, Levamisole, Rabbit traits



[Full text-[PDF](#)] [RiCeST]

## Research Paper

### Dog Bites and Rabies: A Decade Perspective in Nigeria (2005-2014)

Tekki SI, Odita CI, Idachaba ES, Akanbi BO, Moses DG, Barde JI, James AS, Rimfa AG, Kumbish PR, Agama C, Zhakom PN and Okewole PA  
*World's Vet. J.* 6(1): 19-24, 2016;  
pii:S232245681600003-6



#### ABSTRACT

Rabies is a fatal zoonotic encephalitis caused by the rabies virus commonly transmitted to human and other mammals by dog bites. A 10 year review of dog bite cases in humans from 2005 to 2014 was undertaken from archives of the rabies laboratory, National Veterinary Research Institute (NVRI) Vom, Nigeria to assess the magnitude of dog bites and associated risks of human exposure to rabies among bite victims. Of the 1, 840 cases reported, the highest and the lowest rates of bite occurred on 2009 and 2007 respectively. Children constituted 31.5% of the victims, 36.0% were adults, while 32.5% had no age indications. Male victims formed 46.7% of the cases, 38.4% involved females while the genders of the remaining 14.9% were not given. Similarly, prevalence of rabies cases were highest and lowest in 2009 and 2007 respectively while the overall prevalence of rabies-positive dog bite cases during the decade were high (61.1%). However, rabies public campaigns by indigenous veterinary professional groups during the initial editions of the world rabies day improved the level of awareness, which possibly led to the rise in reported cases of dog bites in 2009, while the considerable drop in the cases and probably in rabies in subsequent years, could have been due to vaccination of a considerable number of the dog population. Appreciable reduction in dog bite cases and in rabies nationwide in Nigeria, are only achievable when stakeholders determine to tackle dog bites by supporting responsible ownership and annual mass vaccination of dogs and cats against rabies as well as quarantining or controlling their movements. In rural Africa, where the risk of dog bites and rabies is greatest, it is important to raise public awareness on the roles of accurate laboratory diagnosis and surveillance in the national rabies control and monitoring program.

**Key words:** Dog bite cases, Humans, Rabies, Nigeria

[Full text-[PDF](#)] [[RiCeST](#)]

## Review

### A Review Article of Artificial Insemination in Poultry.

Getachew T.  
*World's Vet. J.* 6(1): 25-33, 2016;  
pii:S232245681600004-6

#### ABSTRACT

The objective of this review is to discuss the history, importance of Artificial Insemination (AI), semen collection and deposition techniques, physiology of cockerel reproduction, characteristics and chemical components of chicken semen, semen storage, and evaluation of semen, semen extenders and behavior of sperm in the oviduct. The first AI in poultry was reported in 1936. All of the avian male reproductive system is inside the bird unlike the males of mammalian species. Avian semen contains energy source to help the viability of semen for AI. For the purpose of in vitro storage semen extenders with appropriate osmolarity and source of energy can be used. Abdominal massage method is the most common technique to collect semen. The technique involves restraining the male and gently stroking the back of the bird from behind the wings towards the tail with firm rapid strokes and gentle squeeze to extract the semen. There are two methods of semen deposition in poultry. These methods are the Intra-peritoneal insemination and vaginal insemination. Vaginal insemination is the commonly used method. It involves everting (turning inside out) the cloaca to expose the reproductive tract of the female.

**Key words:** Artificial insemination, Extenders, Ova, Semen



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## Case Report

### Intestinal Ulceration in West African Mud Turtle (*Pelusios Castaneus*).

Folayemi O-A, Zainab Omolara A, Adenike O-A, Inalegwu Godwin O and Samuel Gbadebo O.  
*World's Vet. J.* 6(1): 34-37, 2016;  
pii:S232245681600005-6

#### ABSTRACT

An adult female West African mud turtle (*Pelusios castaneus*) that had been acquired as part of study on the digestive anatomy on the *P. castaneus* presented mild signs of anorexia that had persisted for a week. On radiographic examination of the digestive tract using Barium sulphate contrast agent, a normal study was observed but an area of contrast coating remained at the region of the duodenum following excretion of the contrast agent. The digestive tract was isolated and gross examination of the coated area revealed areas of ecchymotic hemorrhage and ulcers in this turtle. With not much scientific research available on this wildlife species, this



case of a gastrointestinal tract abnormality is probably the first report of a digestive tract pathology seen in this tropical fresh water turtle.

**Key words:** *Pelusios castaneus*, Duodenum, Ecchymotic hemorrhage, Ulcer

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**ISSN:** 2322-4568

**Frequency:** [Quarterly](#)

**Current Volume:** 6 (2016)

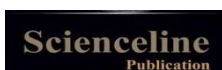
**Current Issue:** [1 \(March\)](#)

**Publisher:** [Scienceline Publication](#)

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Email: [administrator@science-line.com](mailto:administrator@science-line.com)



# Effects of Drugs on Serum Biochemical Profile and Renal Failure in Humans and Experimental Rats: A Preliminary Report

Nabaa Kamal Al shafei<sup>1\*</sup> and Abdelfattah Nour<sup>2</sup>

<sup>1</sup>Department of Biochemistry, Bahri University, Faculty of Veterinary Medicine, Bahri University, Sudan

<sup>2</sup>Department of Basic Medical Sciences, School of Veterinary Medicine, Purdue University, USA

\*Corresponding author's Email: nabaak@yahoo.com

## ABSTRACT

Antibiotics and cancer treatment drugs have been associated with renal failure. In developing countries, antibiotics are often obtained without prescription and this misuse increases the risk of renal failure, especially for patients which have been taking gentamicin for a long-term. For example, taking the aminoglycoside antibiotic, particularly in diabetics, without close medical supervision has been suggested to the cause kidney damage and renal failure in many patients. The objectives of this preliminary study were to investigate the association of antibiotics and renal failure in Hemodialysis (HD) patients in Egypt; and to document the changes in biochemical profiles and histopathology of the kidneys when nephrotoxicity is induced by the antibiotic gentamicin (GM) and the cancer therapeutic agent Cisplatin (Cis). Serum biochemical profiles of HD patients and rats with induced renal failure were compared. Fifty HD patients and six normal people were employed in the study. Sixty experimental rats were divided into three groups: Control, gentamicin-induced renal failure group, and cisplatin-induced renal failure group. The survey of HD patients revealed that 6% of the renal failure is antibiotics related. GM and Cis caused kidney damage and renal failure in rats. The levels of blood serum urea, creatinine, and potassium significantly increased in drug-induced renal failure rats. Serum sodium decreased in GM rats with renal failure and in HD patients compared to the control groups. The histopathological changes in renal tissues in Cis and GM induced renal failure appeared in early stage of renal dysfunction. More studies are needed to determine the correlations between biochemical markers and histopathological changes that may be used as an early warning system for assessing earlier drugs-induced stress to the kidneys before renal failure progresses.

**Key words:** Gentamicin, Cisplatin, Drug-Induced renal failure, Hemodialysis, Serum biochemical profile

## INTRODUCTION

The accumulation of solutes in the blood and tissues is mainly due to inability of the failing kidneys to filter the blood plasma and remove the wastes of metabolism and the unwanted substances. Retained solutes are generally called uremic toxins when they contribute to the deterioration of the physiological and biochemical functions of the kidneys as the renal failure progresses. Therefore, kidney failure or dysfunction causes accumulation of toxins in the body, which result in health complications because of the effects on the blood, the brain, and the heart. These adverse health effects make renal failure a very serious and fatal disease if it is neglected. Several factors, including drugs such as antibiotics, are implicated as causes of renal failure in patients (Heleigh, 2012). The major causes of renal disease in such patients include drug toxicity and glomerulonephritis (Adu and Tse, 2001). The administration of drugs can lead to the deterioration of the renal function. For example, taking aminoglycoside antibiotics, particularly in diabetics, without close medical supervision has been suggested as the cause of renal failure. Moreover, non-steroidal anti-inflammatory drugs (NSAIDs) are considered a major cause of chronic renal failure. Accordingly, it is recommended that such drugs should be avoided or used under strict medical supervision. Perhaps the most widely used drug in the aminoglycosides category is Gentamicin (GM). Apart from their beneficial effects, aminoglycosides induce nephrotoxicity in 10-20% of therapeutic cases. The nephrotoxicity is characterized by tubular necrosis and other structural damage and cell death results in decreased in glomerular filtration (Martínez et al., 2007). In most developing countries, antibiotics are often obtained directly from the pharmacy without prescriptions. On the other hand, Cisplatin (Cis) (cis diamminedichloroplatinum, cis-DDP), a platinum-based drug that has been commonly used in the chemical treatment of various cancers (David et al., 2010), has been implicated as a cause renal failure. Cancer is spreading in developing countries at an alarming rate. It is important to note that irrespective of the insulting agent, the changes that occur as a result renal failure include changes in the serum biochemistry profile, and histopathological characteristics of the failing

ORIGINAL ARTICLE  
 pii: S232245681600001-6  
 Received: 07 Jan 2016  
 Accepted: 20 Feb 2016



kidney (Stordal and Davey, 2007). The objectives of this work was to investigate the association of antibiotics and renal failure in Hemodialysis (HD) patients in Egypt and to document the changes in biochemical profiles and histopathology of the kidneys when nephrotoxicity is induced by antibiotics (gentamicin) and cancer therapeutic agent (Cisplatin).

## **MATERIAL AND METHODS**

### **Experimental animals**

Sixty white male albino rats between the ages of 50-60 days and with mean weight of 190 g were used in this investigation of the present study. Animals were obtained from laboratory animal research Center, faculty of veterinary medicine, Benha University, Benha, Egypt. Rats were housed in hygienic metal cages in the laboratory animal housing facility of the faculty of veterinary medicine of Benha University, Benha, Egypt. Clean and free drinking water and food were supplied ad libitum. Also, the animals in their cages were kept at constant experimental conditions in terms of temperature, humidity, and air pressure, and are provided with optimal nutritional conditions throughout the period of the experiment. Experimental rats were kept two weeks for acclimatization before the beginning of the experiment.

### **Drugs induced nephrotoxicity**

The experimental induction of renal toxicity in male rats was carried out using cis and GM. Experimental rats were randomly assigned to one of three equal groups (20 rats each), placed in individual cages as follows:

Group (1): Normal non-treated rats, served as control group; Group (2): Animals were injected with Cis solution intra-peritoneally (IP), once at a dose of 6mg/kg body weight as described by Bagnis et al. (2001) for induction of renal failure (Cis injected group); Group (3): Animals were injected daily with IP injections of GM at a dose of 80 mg/kg of body weight for eight days as outlined by Abdel-Gayoum et al. (1994) for induction of renal failure (GM injected Group).

### **Blood samples**

In day nine of the experiment, 1 ml blood sample from each rat was collected from the orbital sinus in clean test tubes. Care was taken not to inflict undue stress on the rats, or contaminate the samples. Benha University's Protocol for humane handling of animals was strictly followed. Blood samples were allowed to coagulate at room temperature for 30 minutes, and then centrifuged at 3000 revolutions per minute for ten minutes. The clean serum from each sample were aspirated carefully by a pasture pipette, and transferred into a dry, sterile, and labeled glass vials. The sera were then kept in a deep freezer at -20°C until biochemical analysis of the samples was performed. Four animals from each of the three groups were humanely euthanized and dissected at 48 hours intervals till the end of experiment, following the institutionally approved procedures for humane handling of experimental animals. Serum urea was determined by using the urease- modified Berthelot enzymatic reaction following the procedure of Patton and Crouch (1977). Serum creatinine was determined by using Jaffe Colorimetric end point method according to the method of Houort (1985). Serum sodium was determined colorimetrically according to the method of Trinder (1951). Serum potassium was determined colorimetrically using the turbid metric method according to the procedure described by Terri and Sesin (1958).

### **Tissue samples**

At the 7th day of the experiment and immediately after the rats were euthanized and dissected, the two kidneys were isolated, washed with saline and blotted between filter papers. A half from each of the two kidneys was preserved in 10% neutral formalin in separate labeled jars for histopathological examinations. Samples were dehydrated through ascending grades of ethanol, cleared in xylene, embedded in paraffin wax, and sectioned at 4 - 5  $\mu$  thick. The sections were stained with hematoxylin and eosin, for general histopathological examination as described by Bancroft and Stevens (1977) Crossman stain was used following the procedures outlined by Gray (1954).

### **Survey data analysis of HD patients**

Ninety nine (n=99) chronic HD patients at Benha University hospital, Egypt (Artificial kidney Unit) were identified, fifty patients (n=50) were randomly selected for the study. Six normal people were used as control. The data for this study included survey data of the 50 randomly selected chronic HD patients. The main method of data collection for this study was structured mainly as a closed-ended questionnaire. The questionnaire was made flexible enough so it could be used for patients with different educational backgrounds, and from different economic strata. In an effort not to put the patients under stress while there were going through their regular hemodialysis session, the average interview time was planned not to exceed half an hour. After carefully explaining the goals of the study to each patient, consent was obtained from each patient before the interview. The study was also approved by the hospital board of Benha

University, Benha, Egypt, that made sure that the study would adhere to the guidelines that govern the ethical use of human subjects in research.

### Biochemical profile of serum samples from HD patients

From each of the fifty HD patient, a blood sample for serum separation were drawn during the dialysis in clean test tube from the arterial end of the vascular access of the internal arterio-venous fistula. Samples were also obtained from each of the six normal controls. Blood samples were collected in 3 ml tubes and processed as described earlier for experimental rats. Laboratory assays were performed, using the procedures outlined. On each serum sample, blood urea (Ur), creatinine (Cr), sodium (Na), and potassium (K) were determined.

### Statistical analysis

Data were expressed as means  $\pm$  standard error of the mean, and were compared using the F-test analysis of variance (ANOVA) following the method described by Snedecor and Cochran (1969). Survey data was calculated as percentages and reported in the table 3.

## RESULTS

Serum biochemical profiles of HD patients and rats with induced renal failure were compared. The results of the serum biochemical profiles of the experimental animals and HD patients are presented in table 1 and table 2, respectfully. The summarized survey data of HD patients is presented in Table 3. Figure 1 shows the normal histology of the rats' kidneys. Figure 2 and Figure 3 depict the histopathological changes that occurred in the kidneys following the GM and Cis injections, respectively. Comparisons were made at the 7th day post injections to illustrate the drug-induced renal damage.

**Table 1.** Comparison between serum biochemical profiles in experimental rats

Serum Parameters	Control group (mg/dl)	Cis group in day nine (mg/dl)	GM group in day nine (mg/dl)
Urea	41.06 $\pm$ 3.76 <sup>b</sup>	47.91 $\pm$ 16.05 <sup>c</sup>	422.30 $\pm$ 75.24 <sup>a</sup>
Creatinine	1.06 $\pm$ 0.32 <sup>c</sup>	1.32 $\pm$ 0.10 <sup>b</sup>	3.34 $\pm$ 0.25 <sup>b</sup>
Sodium	143.95 $\pm$ 1.02 <sup>a</sup>	143.98 $\pm$ 3.16 <sup>a</sup>	115.32 $\pm$ 8.47 <sup>b</sup>
Potassium	5.89 $\pm$ 0.46 <sup>b</sup>	4.71 $\pm$ 0.30 <sup>b</sup>	8.52 $\pm$ 0.26 <sup>a</sup>

Data are presented as mean  $\pm$  standard error. Means values with different superscripts in the same row are significantly different (P<0.05)

**Table 2.** Comparison between serum biochemical profiles in control and HD patients, Benha University Hospital, Benha, Egypt (2011)

Serum Parameter	Control (mg/dl)	HD Patients (mg/dl)
Urea	20.5 $\pm$ 4.45 <sup>a</sup>	83.67 $\pm$ 21.67 <sup>b</sup>
Creatinine	0.74 $\pm$ 0.15 <sup>a</sup>	5.08 $\pm$ 1.69 <sup>b</sup>
Sodium	120 $\pm$ 24.09 <sup>a</sup>	71.67 $\pm$ 16.65 <sup>b</sup>
Potassium	3.91 $\pm$ 0.8 <sup>a</sup>	7.46 $\pm$ 2.42 <sup>b</sup>

Data are presented as mean  $\pm$  standard error. Means values with different superscripts in the same row are significantly different (P<0.05)

**Table 3.** Frequency of the cause of renal disease in HD patients, Benha University Hospital, Benha, Egypt, 2011.

Causes of renal disease	Frequency
Renal hypertensive disease	18 (36%)
Diabetic nephropathy + renal hypertensive disease	8 (16%)
Uremia, etiology unknown	7 (14%)
Pre-eclampsia	5 (10%)
Rheumatoid arthritis	3 (6%)
Treatment with antibiotics	3 (6%)
Kidney calculi (nephrolithiasis)	2 (4%)
Polycystic Kidney disease	2 (4%)
Diabetic nephropathy	1 (2%)
Severe anemia	1 (2%)
Total	50 (100%)

HD: Hemodialysis patients. Data from all patients were pooled and percentages calculated and displayed in the present table,

From Table 1, when rats are compared at 9th weeks after drug-induced renal failure, it could be observed that urea levels in the serum increased compared with the control. However, the increase is more pronounced in the GM rats. On the other hand, creatinine also increased and this is similar to that observed for urea. Sodium significantly (P<0.05) decreased in the GM group but not in the Cis group. Potassium showed a significant increase in the GM group compared to the control. However, potassium in the Cis group was not statistically different from that of the control group.

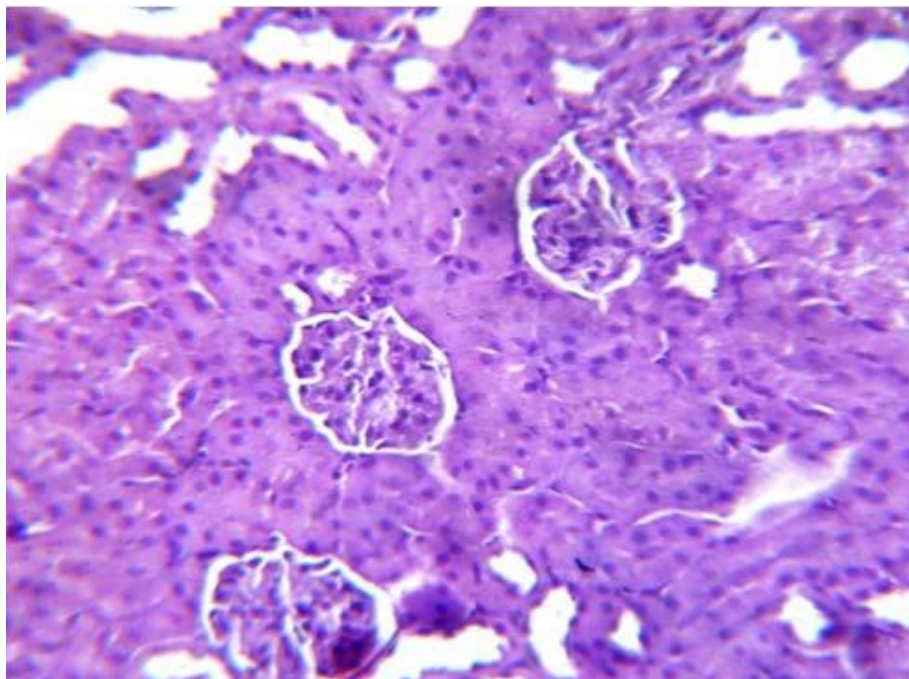
Table 2 presents the data for serum urea, creatinine, sodium and potassium in normal and HD patients. Similar to what observed for drug-induced renal failure in rats, urea and creatinine increased in HD patients compared to the control. Sodium significantly decreased in HD patients, while potassium significantly ( $P < 0.05$ ) increased. The trend in the serum biochemical profile observed in GM- induced renal failure is similar to that of HD patients.

### Macroscopic and microscopic appearance

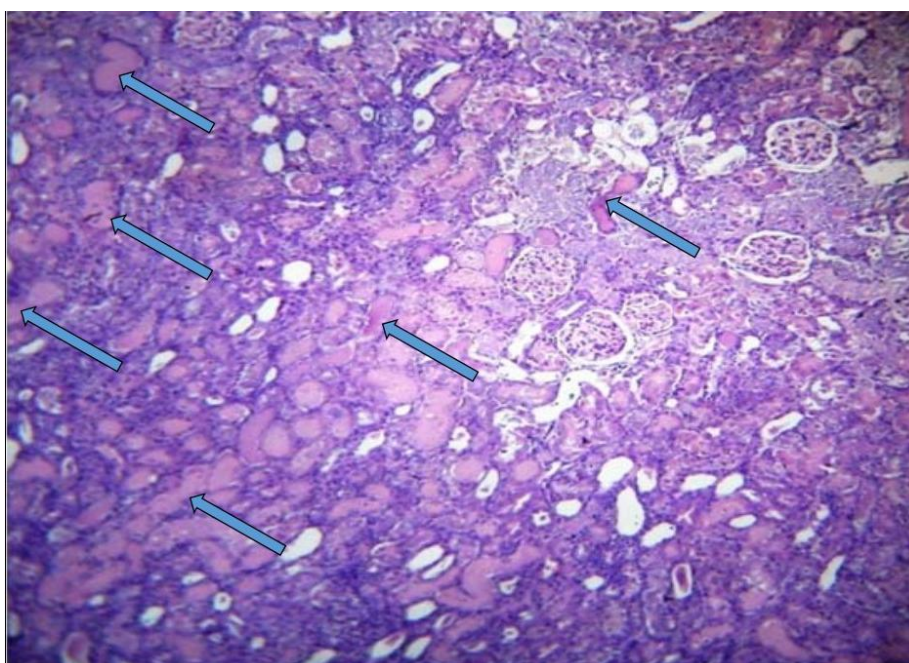
Macroscopically, the kidneys of experimental animals were enlarged in size, pale in color, and edematous or soft in consistency. The cut section also was pale in color. Microscopically, the kidney sections obtained from the control groups show a normal histological structure (Figure 1).

The hyaline cast in lumina of renal tubules in shown in figure 2 for kidneys obtained from the rats at seventh day post GM injection. Also shown, is the cell infiltration and vacuolation in their cytoplasm (Figure 2).

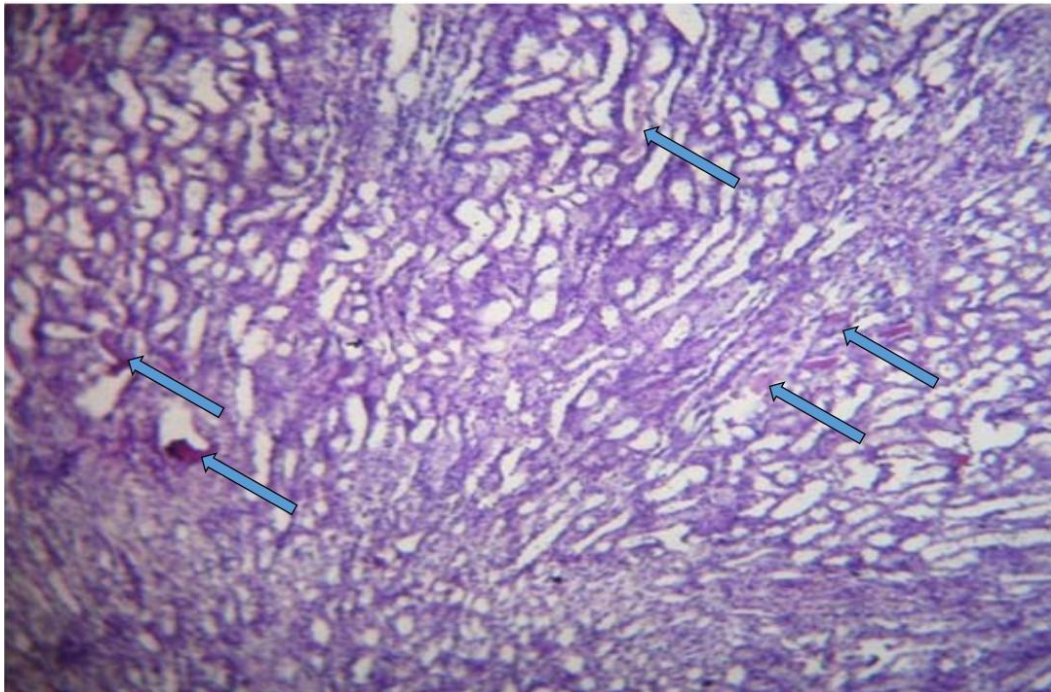
Figure 3 shows that at day seven of the Cis group, there was necrotic cellular debris in the lumina in some renal tubules, and others contained albuminous granules. Other tubules showed cloudy swelling.



**Figure 1.** Kidney of normal adult rat (renal cortex) shows normal histological structure with H & E stain under light microscope (100X)



**Figure 2.** Kidney of Gentamicin-induced rat at day 7 shows hyaline cast (arrows) in renal tubules in their Lumina with H&E stain under light microscope (100X)



**Figure 3.** Kidney of Cisplatin-induced rat at day 7 shows renal tubules contain hyaline cast (arrows) with H&E stain under light microscope (100X)

### Survey of HD patients

In this study, the survey data provided information of the possible causes of chronic renal failure. Background information of HD patients was reported by Alshafie et al. (2011). Table 3 summarizes the possible causes of renal failure in the 50 HD patients randomly selected for this study. From this table it could be observed that over dose or uncontrolled or long-term treatment with antibiotics seems to have caused renal failure in 3 (6%) of patients in the present study.

### DISCUSSION

In the experimental rats, the occurrence of drug-induced nephrotoxicity was documented in the Cis and GM groups by many criteria including: significant number of deaths among the rats during the experimental period, 13 rats out of 20 (65%) in the GM group (Alshafie et al., 2011). There was a significant ( $P < 0.05$ ) increase in serum Ur, Cr and potassium levels (Table 1). These results are in agreement with Daniel (1998) who concluded that the increased death rate in case of GM treated rats is due to uremia, hyperkalemia, septicemia, and gastrointestinal hemorrhage.

### Cisplatin

The marked increase (Table 1) in serum Ur, and Cr levels in Cis treated group compared to the control group were in agreement with Arhoghro et al. (2012) and Saumya et al. (2011) who observed that, Cis injected rats had encountered acute kidney dysfunction as evidenced by elevation in serum Ur, Cr and uric acid decreased urine output and body weight with multiple histological damages. As mentioned earlier for GM the drug-induced histopathological changes are important causes of renal failure as shown in figure 2 when comparisons were made with the control group at the 7th day of the experiment. The histopathological changes that occurred at 3rd, 9th, and 11th days were reported by Alshafie and Nour (2011). Moreover, Khoshnoud et al. (2011) showed that, serum Cr and Ur levels were significantly elevated ( $P < 0.05$ ) in the animals treated with Cis compared to the normal control group. The increase of serum Ur and Cr levels were 6.5 and 5.8 folds, respectively. Jisha et al. (2008) as well, documented in Cis treated group a similar increase in serum Ur and Cr. Cis treatment resulted in a two fold increase in the serum urea and creatinine levels as compared to that of the untreated control group. The results of this study also agreed with the findings of Zhanjun et al. (2011) who concluded that mice treated with Cis showed earlier and greater increases in the levels of Ur and serum Cr. Furthermore, Udeani et al. (1996) observed a decline in renal function four days after Cis therapy, with a concomitant increase in serum Cr and blood Ur concentrations. Conklin (2000) mentioned that evidence suggests Cis-induced nephrotoxicity is due to the reactive oxygen species and the renal antioxidants. Ayman et al. (2006) indicated that, kidney damage induced by Cis treatment was characterized by alterations in kidney function, as evidenced by the continuing increase in serum Cr and blood urea nitrogen (BUN) levels. Interestingly, serum Cr increased significantly than BUN after Cis treatment, which may be due to the acute nephrotoxicity as suggested by Arunkumar et al. (2011). Furthermore, Cis-induced renal

oxidative stress, the inflammatory response, and the histopathological injury had led to renal dysfunction (Mukhopadhyay et al., 2012).

The serum sodium level of Cis injected group in this study (Table 1) was different from had found by Peyrade et al. (1997), who concluded that Cis treatment resulted in hyponatremia that requires specific management. Also, Shafaq and Tabassum (2011) mentioned significant decrease in plasma sodium level of CIS treated rats when compared to the control group, the changes in the antidiuretic hormone secretion have been reported as the underlying mechanism for Cis chemotherapy induced hyponatremia (Lequn et al., 2002). Furthermore, hyponatremia might be attributed to the effects of the circulating uremic toxins, which resulted in accumulation of sodium inside the cell and loss of potassium as stated by Kosa and Silva (1980). Cis treatments can lead to hyponatremia in 4 to 10% of cases due to salt wasting with hypomagnesemia and normokalemia. In addition, functional renal failure and associated hyponatremia and hypotension were observed by Peyrade et al. (1997). Our study did not observe hyponatremia in Cis-treated rats.

The increase in serum potassium levels observed in experimental group in this study (Table 1) is consistent with the findings of Marshal (1988). Marshal (1988) documented the presence of a hyperkalemia after the progression of renal failure, and attributed that to the decreased excretion of potassium. Moreover, Lajer et al. (2005) concluded after repeated Cis treatments in rats that, Cis treatment exerted a negative effect on total potassium balance, although the difference between Cis-treated and the control rats was not significant at the end of their follow up. However, Shafaq and Tabassum (2011) reported a non-significant increase in serum potassium level in Cis treated rats as compared to the control. Surgam and Kahn (1986) explained that hyperkalemia might be related to the secondary hyperparathyroidism following uremia, and could be due to impairment renal excretion of potassium. Soliman et al. (1989) observed that the administration of parathyroid hormone to experimentally uremic rats caused a marked rise in plasma potassium level. In contrast to these findings, Khoshnoud et al. (2011) argued that Cis injection at a dose of 5 mg/kg did not change serum potassium levels significantly when compared with control group. Lajer and Daugaard (1999) argued that electrolyte disturbances such as hypomagnesaemia (60%), hypocalcaemia (89%), hypophosphatemia (57%), hypokalemia (95%) and elevations in serum Cr and Ur were observed after Cis-based chemotherapy. Our results agree with those reported in the literature in that Cis treatment resulted in a decrease in serum potassium.

### **Gentamicin**

GM is a widely used antibiotic against serious and life-threatening infections, but its usefulness is limited by the development of nephrotoxicity (Randjelovic et al., 2012). Drug-induced nephrotoxicity is an important cause of renal failure as shown in Figure 2 when comparisons were made with the control group at the 7th day of the experiment. The histopathological changes that occurred at 3rd, 9th, and 11th days were reported by Alshafie and Nour (2011). The nephrotoxicity produced by GM can be due in part to an imbalance of pro-oxidant and antioxidants (Poormoosavi et al., 2010). The significant increase in serum Ur and Cr observed in this study (Table 1) is in agreement with that found by Marwan and Afaf (2012) in that GM produced highly significant ( $P < 0.001$ ) increase in the concentration of serum Ur and Cr and confirmed that GM produced nephrotoxicity. Furthermore, Poormoosavi et al. (2010) showed that the administration of GM to rats once daily for seven days reduces glomerular function, as reflected by the increased serum Ur and Cr concentrations. The increase in serum Ur and Cr levels were documented in the GM treated group compared to the control rats, were also in agreement with Freddy et al. (2009) who demonstrated that the mean serum Cr and Ur levels had significantly increased, after nine days of treatment with GM in comparison to that of the control group. Moreover, Patil et al. (2010) used GM at a dose of 100 mg/kg in rats and reported similar results to those observed in this study. Administration of GM alone at a dose of 100 mg/kg/day for eight days resulted in an increased level of serum Cr with evident nephrotoxicity (Eldin et al., 2008). Shaibu and Aminu (2009) confirmed the increase in serum levels of Cr and Ur after intramuscular injections of GM at a dose of 80 mg/kg body weight/day produced nephrotoxicity, as evidenced by the continuing increase in serum Ur and Cr. This was also confirmed by Nasim et al. (2006) who showed that rat groups injected subcutaneously with GM for seven days, euthanized on days 8 and 15, revealed highly significant ( $P < 0.001$ ) increases in Cr and Ur serum levels and histological change suggestive of nephrotoxicity. Nenad et al. (2008) observed that, aminoglycoside antibiotics are toxic to the kidneys and would result in renal failure during prolonged use, and the experimental models of GM-induced nephrotoxicity have shown histopathological, ultra structural and functional alteration with BUN, uric acid and serum Cr increase leading to acute renal insufficiency. This is consistent with the findings on this study. Nagai (2006) mentioned that, as aminoglycosides are taken up into the cells of the renal proximal tubules, and because they remain in these cells for a long time, result in nephrotoxicity. The GM damage was believed to be related to the generation of Reactive Oxygen Species (ROS) in the kidney (Martínez et al., 2002). ROS induces vasoconstriction, decreases Glomerular Filtration Rate (GFR) and also induces cellular injury and necrosis via lipid peroxidation and protein modification (Edson and Terrell, 1999).

The decrease in serum sodium levels observed in Table 1 in the GM-treated group compared to the control rats, is similar to the findings of Francescato et al. (2012) who mentioned that, rats injected with GM for nine days and

ethanized two days after the end of this treatment, presented a higher plasma Cr and Ur levels, and increased sodium excretions. Also, Marwan and Afaf (2012) showed that serum electrolytes had significantly ( $P < 0.001$ ) changed in GM-treated rats as compared with control animals. Lower value of serum sodium indicates the inability of the kidney to conserve sodium and chloride. Hemodilution too may be involved in the decrease of serum sodium levels via the excess of water intake, and or increased production of metabolic water (Marwan and Afaf, 2012). Moreover, Poormoosavi et al. (2010) documented that serum sodium level had significantly decreased in GM group, however the serum potassium level significantly increased in GM group. On the other hand, Nenad et al. (2008) observed no statistically significant differences between the values of serum sodium concentrations in the GM group and the control group. The observed hyponatremia in the experimental groups treated with GM might be due to increased aldosterone production which affects the intracellular distribution of sodium potassium pump, leading to an increase of potassium and a decrease of sodium in tissues in uremic patients (Akmal et al., 1985). Another suggestion was advanced by Massary (1983) in that the decreased serum sodium level could be related to the metabolic acidosis accompanied uremia. The decreased serum sodium level in this study was not in agreement with the findings of Khalida et al. (2011) who mentioned that level of serum sodium had slight ( $P > 0.05$ ) increased in GM group, whereas level of serum potassium had significantly decreased in groups of rabbits receiving GM compared to the control group. The differences between experimental animals might have lead to the different results.

Regarding hyperkalemia observed in this experiment (Table 1) our results were in agreement with Poormoosavi et al. (2010) who mentioned that GM-induced nephrotoxicity is characterized by a decrease in the glomerular filtration rate, hyponatremia, hyperkalemia, and direct tubular injury. Moreover, Weinberg et al. (2009) stated that the risk of hyperkalemia in GM group is small. The increases of potassium appeared to be due to reduced excretion of potassium aggravated by leakage of intracellular potassium into blood stream as a result of GM- induced damage to the renal tubular epithelium (Marwan and Afaf, 2012). In contrast with these findings, Afzal et al. (2012) recorded a significant fall in serum potassium levels on day 16 in the animals treated with GM compared to the group treated with pyridoxal phosphate, where potassium levels remained similar to that observed on the First day. Khalida et al. (2011) stated that the administration of GM resulted in a significant decrease in serum potassium throughout the experiment. However, Enver et al. (2003) argued that in the GM-induced nephrotoxicity in experimental rats, potassium and sodium levels were altered by the treatment.

### **Survey of hemodialysis patients**

The survey data revealed that 6% of the HD patients had renal failure that was attributed to antibiotics. Taking GM, particularly in diabetics, without close medical supervision, and the consumption of NSAIDs are being considered major causes of chronic renal failure. Because of the risk involved, it is recommended that taking such drugs should be avoided, or if necessary taken under strict medical supervision, which does not usually happen in developing countries, especially Africa. Adu and Tse (2001) suggested that the major causes of renal disease in such patients include drug toxicity and glomerulonephritis. Acynthia (2008) concluded that, drugs are a common source of acute kidney injury. The results obtained in this study are in agreement with the above mentioned investigations. The possible association of rheumatoid arthritis and chronic renal failure observed in this study is in agreement with Karstila et al. (2007) who reported that the occurrence of rheumatoid arthritis has varied between 5 and 50 percent in patients with chronic kidney disease which might be related to NSAIDs and antibiotics treatment. Drugs are shown to cause nephrotoxicity and exert their toxic effects by one or more common pathogenic mechanisms. However, Gilbert (1991) observed that, despite the mechanism of drug induced renal failure, the drug is actively concentrated in the renal cortex and proximal tubular cells where it reaches maximum concentration. Hence, high drug concentrations or multiple dosing are expected to be more harmful to the kidneys. This is of particular concern in countries of Africa where the lack of knowledge of the harmful effects of drugs, and the ease with which antibiotics are obtained might have resulted in drug toxicity and the associated kidney failure.

### **CONCLUSION**

Several factors and drugs, such as antibiotics and a variety of other pharmaceutical agents, are known to be toxic to the kidneys. Renal toxicity commonly occurs after administration of these nephrotoxic agents. Nephrotoxicity has been listed as one of the major side effects of the drug GM, whose plasma half-life is increased when the glomerular filtration rate is low. On the other hand, a single dose Cis injection may cause impairment of renal functions and cause severe histopathological injury that would lead to the development of renal failure. The traditional measures of renal damage, elevated Cr and Ur levels usually occur after significant kidney damage has taken place. This study showed that the histopathological changes in renal tissues in Cis and GM-induced renal failure appeared in early stage of renal dysfunction in comparison to the changes in serum levels of traditional biomarkers such as urea and creatinine. More

studies are needed to determine the correlations between biochemical markers and histopathological changes that may be used in prediction equations to assess earlier the drugs-induced toxicity in the kidneys before renal failure.

### Acknowledgements

We would like to express our sincere gratitude to the Egyptian patients who signed the consent forms and participated in the study. Thanks are also extended to the staff of Artificial Kidney Unit and the clinical pathology lab of Benha University's hospital, Egypt, for their valuable help. Gratitude and thanks are also due to the staff of the Animal House for their help in taking care of the experimental animals.

### Competing Interest

The authors declare that there are not significant personnel, professional or financial competing interest that might have influenced the presentation of the results of the study described in this manuscript.

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# Evaluation of Using Honey, Cool Water and Levamisole against Heat Stress on Different Traits of Rabbits under Egyptian Summer Conditions

Nagham Rafeek El Saïdy<sup>1\*</sup>, Fatma, El-Sayed Allam<sup>2</sup>, Tarek Mousa Balabel<sup>3</sup> and Sami Abdel-Aziz El-Midany<sup>1</sup>

<sup>1</sup>Department of Hygiene and Preventive Medicine, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh Governorate, Egypt.

<sup>2</sup>Post Graduate Student at Department of Hygiene and Preventive Medicine, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh Governorate, Egypt.

<sup>3</sup>Department of Hygiene and Preventive Medicine (Animal Behavior and Welfare), Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh Governorate, Egypt.

\*Corresponding author's Email: nagham.elsaidy@yahoo.com

## ABSTRACT

This study was conducted in order to estimate the impact of using honey in drinking water, drinking cool water and Levamisole injection as alleviated tools of heat stress on White New Zealand rabbits under Egyptian summer conditions. 40 sexually mature White New Zealand rabbits contained 36 does with an average age of 15-20 (18±2) weeks and nearly similar body weight of 2 kg and 4 fertile bucks with an average age of 24 weeks and average weight of 2.5 kg were used in this experiment. They were allocated into four groups each containing 9 does and 1 buck. Group I was kept as a control without any treatment, group II received honey 20 ml/l on drinking water, group III drank cold water with a temperature ranged from 16-20 °C and group IV received a single dose of subcutaneous Levamisole injection (2 mg/kg BW). Results showed that most of performance, reproductive and physiological parameters of examined rabbits were significantly decreased by heat stress under Egyptian summer conditions. However, treated groups showed improved traits on most examined parameters comparing with control group. Among the treated groups, the one treated with honey expressed significant increase (P<0.05) in body weight, body weight gain, feed intake, feed conversion ratio, water consumption, conception rate, litter size and weight, milk yield, economic efficiency, rectal temperature, hematological parameters and some serum biochemical parameters. On the other hand, the group which received cool water showed the best records for decreased levels of serum urea, creatine and respiratory rate. In conclusion, it is clear that heat stress has negative effects on reproductive and physiological traits of growing rabbits with drawing attention toward the importance of using alleviating methods for mitigating the negative effects of heat stress especially by using honey and drinking cool water.

**Key words:** Heat stress, Honey, Cool water, Levamisole, Rabbit traits

## INTRODUCTION

Commercial rabbit production has been gaining much attention in recent years due to its high prolificacy, rapid growth rate, small body size and high meat yields (Marai et al., 1999). Rabbits can convert 20% of protein they eat into edible meat which is higher than pigs (16-18%) and beef (8-12%) (Basavaraj et al., 2011). Heat stress is defined as a stress inflicted by a wide range of environmental conditions that induce a state of physiological problems within animal's body, makes animals unable to regulate their heat homeostasis passively (Askar and Ismail, 2012). Heat stress is the most obvious limitation to rabbit production in regions with a hot climate (Ondruska et al., 2011).

Egypt climate is characterized by a long hot period (from May to October); in this period rabbits have difficulty in eliminating their body heat due to their nonfunctional sweat glands (Marai et al., 1994a, 1994b and 1996). Thermal Neutral Zone (TNZ) temperature in rabbits is around 18–21 °C (Marai and Habeeb, 1994; Habeeb et al., 1998). Keeping the growing animals under high ambient temperature (above 30°C) deleteriously affects their growth performance traits (Habeeb et al., 1992). Furthermore, disturbances in feed intake, feed utilization, water metabolism, blood parameters, and enzymatic reactions, hormonal secretions, in addition to protein, energy and mineral imbalances have been also reported to be disrupted in heat stressed rabbits (Okab and El-Banna, 2003; El-Banna et al., 2005; Burnett et al., 2006 and Okab et al., 2008). Additionally, heat stress negatively affects rabbit's production (Fouad, 2005). Many attempts have been established in order to overcome the detrimental effects of heat stress on growing rabbits, via modifying environmental condition through nutritional, managerial, and physiological manipulation of rabbits (Selim et al., 2003). Moreover, many additives are recently added to rabbit feed or water as a way to help alleviate adverse effect during summer months in a trial to keep the animal within the range of its thermo neutral state that realize comfort, as well as enhance productive performance and immune response of rabbits. Honey is a sweet liquid made by bees using nectar

ORIGINAL ARTICLE  
pII: S232245681600002-6  
Received: 11 Feb 2016  
Accepted: 18 Mar 2016

from flowers, it has high levels of monosaccharides, fructose and glucose, containing about 70 to 80 % sugar, which gives its sweet taste, minerals and water make up the rest of its composition (Graham, 1992).

Honey possesses antiseptic and antibacterial properties. Several studies have shown bee venom to exert both an anti-inflammatory effect, a property shared with non-steroidal anti-inflammatory drugs (Jang et al., 2003), and an antibacterial effect with no side effects in animal models (Han et al., 2006). Furthermore, apitherapy using live honeybee stings had therapeutic value for pigs with respiratory diseases such as atrophic rhinitis, pleuropneumonia, and Glasser's disease (Choi et al., 2003). In addition, providing cool water was found to be effective in alleviating the heat load of rabbits (Abd El Monem et al., 2013). Treatment of heat-stressed rabbits by drinking cool water (10-15 °C) showed the highest body weight, total body gain and margin percentage, as well as improved feed intake, feed conversion and decreased water intake (Marai et al., 1999).

Levamisole is a synthetic imidazothiazole derivative which is a highly acceptable antinematodal drug because of its broad range of activity in a large number of hosts (El-Boshy and El-Deean, 2013). Treatment of heat stressed rabbits by Levamisole showed a return in the increased RBCs count and stress leukogram picture to normal (El-Boshy and El-Deean, 2013). The drug is well absorbed and widely distributed and can be detected in all tissues and fluids with the highest levels in liver and kidneys (Madani et al., 2010). To our knowledge using the honey as a mitigating tool to alleviate the effect of heat stress on rabbits, as well as the effect of using cool water and Levamisole on some reproductive traits of heat stressed rabbits has not been investigated previously. Therefore, the objective of the present study was to investigate the effect of adding drinking water with honey, drinking cool water and injection of Levamisole on some reproductive, performance and physiological parameters of White New Zealand (WNZ) rabbits under Egyptian summer conditions.

## **MATERIAL AND METHODS**

### **Ethical approval**

Animal ethics committee, faculty of veterinary medicine, Kafr-Elsheikh University, Egypt, approved the protocol and conducting of the study.

### **General layout of experiment**

This study was carried out for 9 weeks (from 19th June till 21th August, 2015) at the laboratory of the department of hygiene and preventive medicine, faculty of veterinary medicine, Kafr El-Shiekh University, Egypt.

### **Animals and husbandry**

40 sexually mature WNZ rabbits 36 does with an average age of 15-20 (18±2) weeks and nearly similar body weight 2kg and 4 fertile bucks with an average age of 20-26 (24±2) weeks and average weight of 2.5 kg, which were proven to be fertile, were used in this experiment. Rabbits were individually housed in metal hutches of a commercial type (60×55×40 cm) provided with separate facilities for feeding, watering and nest box (40×30×30 cm). Rabbits were vaccinated by:

1. Cunipravac RHD (inactivated vaccine against hemorrhagic disease, HIPRA company) (0.5 ml S/C).
2. Formalized polyvalent rabbit pasteurellosis vaccine (Veterinary Serum and Vaccine Research Institute. Cairo, Egypt) (2ml S/C).
3. Ivermectin 1% against Mange (Memphis for Pharmaceuticals and Chemical Industries, Egypt) (2ml S/C).
4. Cages and nest boxes were cleaned regularly and disinfected before each kindling. Dropped urine and feces on rabbitry's floor were cleaned every day in the morning. All rabbits were reared under the same managerial conditions. All rabbits were kept under identical hygienic and environmental conditions.

### **Feeding system**

All rabbits under experiment were offered a commercial ration pellets (Super visor Company, Egypt). All nutritional requirements of rabbit does were provided according to National Research Council (NRC) (1994). The chemical analysis of the pellets was carried out according to Association of Official Analytical Chemists Official (A.O.A.C., 1990) it contained 18% crude protein, 10.19% crude fiber, 2.8% crude fat and 2635 kcal/kg diet. Rabbits were fed an amount of pellet ration that provided normal growth and maintained adult body weight. The diet and drinking water were provided twice daily at 9 a.m. and 5 p.m.

### **Climate data**

The climatic data was continuously recorded among the experimental period using thermo hygrometer the weekly averages of ambient temperature and relative humidity values at midday inside the rabbit building were estimated. The

Temperature Humidity Index (THI) was computed using the formula cited by Marai et al. (2001) for rabbits as following:

$$\text{THI} = \text{db } ^\circ\text{C} - \{(0.31 - 0.31 \text{ RH}) (\text{db } ^\circ\text{C} - 14.4)\}$$

Where db °C = dry bulb temperature in degrees Celsius and

RH = relative humidity expressed in percentage

Obtained values of THI classified as follows: absence of heat stress (<22.2), moderate heat stress (22.2-< 23.3), severe heat stress (23.3-< 25.6) and very severe heat stress (25.6 and more) (Abd El-Moneim et al., 2013).

### Experimental design

The rabbits were randomly allocated into four equal groups (n=10) (9 does and 1 buck) as shown in table 1, and housed in separate rooms under the same environmental temperature ranged from 33-36±2 °C and relative humidity (64-79±3 %) (Balabel, 2004). All does were identified and individually mated by transferring each one to the buck cage and returned to their own hutch after copulation. Each doe was palpated 10 days post mating and if there was no pregnancy, she rebred until she got pregnant. On the 27<sup>th</sup> day of pregnancy, the nest boxes were prepared for kindling and supplied with wood sawdust to provide a warm nest for the bunnies. Kits were weaned at 28 days of age. Light/dark rate during the experimental period was 12/12 hours as suggested by Lebas et al. (1984).

**Table 1.** Description of the experimental WNZ rabbits groups used in the experiment under Egypt summer conditions, 19 June- 21 August, 2015.

Groups	Treatments
I	Kept as a control group without treatment
II	Supplemented by drinking water with Honey 20 ml/L
III	Drank cold water (temperature ranged from 16-20 °C)* by adding measurable ice cubes to water from 11 a.m.
IV	Injected subcutaneously by Levamisole (2mg/kg body weight) once**

\*Selim et al. (2004); \*\* (According to the instruction of the producer Company, Memphis for Pharmaceuticals & Chemical Industries, Egypt)

### Measured parameters

**Performance parameters:** Initial and final body weights (Kg) weights were recorded individually weekly throughout the experiment period. The feed consumption (gm/day) and water intake (ml/day) were recorded during the experimental period by weighting the amount of feed and measuring the amount of water remained, then subtracting them from the offered amount before putting the new one. Body weight gain (gm) for adult and young rabbits was calculated as difference between the initial weight and weight after 9 weeks. Feed Conversion Ratio (FCR) was calculated as the amount of food consumed for production of one unit of body gain (Marai et al., 2006). Performance index (PI) was calculated according to Yassein et al. (2008). Mortality rate for does and kits from birth till weaning was recorded.

**Reproductive parameters:** The conception rate was calculated as results of pregnancy diagnosis at first mating attempt for does that had accepted the male (Ahmed et al., 2005). Gestation period was recorded by counting the days between mating and kindling day. Amount of milk yield for each doe during the first 3 days post kindling was recorded according to Cowie (1969). Litter weight and size of total kits born and individual kit weight at kindling and weaning. Fetal losses and dead kits before weaning were recorded.

**Physiological parameters:** Blood samples were collected on a weekly basis, in the morning from the marginal auricular vein from 3 rabbits of each group, into 2 test tubes, the first tube containing EDTA for hematological analysis. Second tube was the centrifuge tube, left to clot and centrifuged to obtain clear serum for serum biochemical analysis kept in a deep freezer at -20°C until analysed (Schalm et al., 1975).

**Hematological parameters:** Whole blood samples were analyzed shortly after collection for measuring Red Blood Cell (RBC) counts, Packed Cell Volume (PCV), total and differential leukocytic count (Coles, 1986). Hemoglobin was determined by colorimetric method (Schalm et al., 1975).

### Serum biochemical analysis

Serum samples were used for measuring serum total protein, urea, creatinine and glucose by using commercial kits (Diamond Diagnostics, Egypt). Globulin was determined by subtracting albumin from total protein (Schalm et al., 1975).

### Respiration rate and rectal temperature

The respiration rate was recorded by counting the flank movements per minute by using a hand counter. The rectal temperature was measured by using a clinical thermometer inserted into the rectum for 2 minutes at depth of 2 cm (Marai et al., 1999).

### Economic efficiency

Economic Efficiency (EE) was calculated as the ratio between income (price of weight gain) and cost of feed consumption during the experimental period according to Abd-Ella et al. (1988).

### Statistical analysis

Data were tested for distribution normality and homogeneity of variance. Data was reported as mean  $\pm$  standard error of the mean and analyzed by ANOVA using SAS (Statistical Analysis Software), Institutes INC (2005). The significance of difference among the different treatments was evaluated by Tukey's test. The significance level was set at  $P < 0.05$ .

## RESULTS AND DISCUSSION

Results at Table 2 show that temperature-humidity index values estimated exceeded than 25.6, indicating exposure of the animals to sever heat stress during the hot period. The results are in the same line of earlier findings of Marai et al. (2000) and Abd El-Moneim (2001) under the same Egyptian climate conditions.

**Table 2.** Average weekly climate data during the experiment period under Egypt summer conditions, 19 June-21 August, 2015.

Weeks	Temperature ( $^{\circ}$ C)	RH (%) **	THI*
1	34.2 $\pm$ 1.1	64 $\pm$ 4	30.6
2	34.6 $\pm$ 1.5	68.1 $\pm$ 2.9	30.6
3	34.6 $\pm$ 0.97	74.8 $\pm$ 2.3	31.1
4	35.1 $\pm$ 1.77	73.4 $\pm$ 3.5	32.2
5	35.2 $\pm$ 1.49	76 $\pm$ 5.1	32.2
6	34 $\pm$ 0.81	74.2 $\pm$ 2.6	31.1
7	34.1 $\pm$ 1.06	66.3 $\pm$ 1.7	30.6
8	34.7 $\pm$ 1.38	73.1 $\pm$ 4.4	31.1
9	35.8 $\pm$ 1.21	74 $\pm$ 2.82	32.2

Mean $\pm$  standard deviation; \*THI: Temperature humidity index; \*\*RH: Relative humidity.

For judging the effect of heat stress and its alleviation that could directly or indirectly affect rabbit's performance, different live performance parameters were studied as an indicator of rabbit's welfare. The performance parameters (final body weight, body weight gain, feed intake, feed conversion ratio and water consumption) which are represented at table 3, indicated that most of growth performance traits studied on examined rabbits were inversely and significantly ( $P < 0.05$ ) affected by heat stress, as it was cleared that the control group (Group I) recorded the lower values for most of performance parameters comparing with treated groups II, III and IV. These results were in agreement with Ayyat et al. (2004) and Villalobos et al. (2008). However, addition of honey in the drinking water of the heat stressed NZW growing rabbits up to 20 mg/l water, significantly ( $P < 0.05$ ) improved most of traits concerning growth performance especially Body Weight Gain (BWG) and FCR, also this group expressed higher numerical values for final body weight, Performance Index (PI) and water consumption. The results are in coincide with Bonomi et al. (2001) who observed weight gain improvement of 11% and 15% and feed utilization improvement by 8.5% and 12.5% when royal jelly (RJ) (a honeybee secretion) was used in rabbits feeding at 15 and 20 ppm from 30 to 90 days of age, respectively. Furthermore, Han et al. (2009) also indicated a net increase in BWG and survivability in piglets with honey venom injection. Moreover, there is the finding of Bonomi (2003) who reported 11% and 14% improvement in weight gain and 5% and 7% improvement in feed utilization when pigs were fed RJ added to mixed feeds at doses of 30 and 50 ppm respectively. However, in the case of feed intake the treated group with cool water recorded the higher numerical values comparing with other treated groups and control group. These results were in agreement with Marai et al. (1999) and Abd El-Moneim et al. (2013) who reported that drinking cool water leads to increase in animal appetite and physiological functions. The findings that could be explained on the basis of that drinking cool water acts through cooling the animal body core by conduction as a result to the difference between temperatures of the drinking water and urine, mediated by cooling the area of the hypothalamus (Abd El-Moneim et al., 2013). However still honey treated group was much better in feed conversion ratio due to higher body weight gain in comparison with the amount of feed intake.

Finally, the Mortality Rate (MR) was 55% in stressed untreated group and decreased at treated groups where the honey treated group expressed lowest MR (11.2%) followed by cool water treated groups (33%) and finally the Levamisole treated group (44%). The results are in the same line of Marai et al. (2002) and Balabel (2004) who reported that rabbits are very sensitive to heat stress since they have few functional sweat glands which means they have

difficulties in eliminating excess body heat when the environmental temperature is high. Habeeb et al. (1997) reported that MR from birth up to weaning was significantly ( $P < 0.05$ ) increased in response to a temperature which increased from 19.5 °C in January to 34.8 °C in July. In addition, the same authors estimated MR in adult rabbits in summer to be 18%, while no mortality was recorded during winter. Data presented at table 3, cleared that all treatment groups improved economic efficiency during the whole experimental period in comparing with control group. It was clear that, the highest value was achieved with honey treated group followed by cool water treated group and finally Levamisole treated group. These results were in accordance with those of Marai et al. (1999).

**Table 3.** Performance traits and economic efficiency % of White New Zealand rabbits under Egypt summer conditions, 19 June-21 August, 2015.

Examined items	Examined groups			
	Control group	Honey treated group	Cool water treated group	Levamisole injected group
Initial body weight (gm)	2.039±0.024	1.973±0.045	2.019±0.041	2.059±0.032
Final body weight (kg)	2.148±0.02778 <sup>a</sup>	2.546±0.05787 <sup>b</sup>	2.495±0.05643 <sup>b</sup>	2.459±0.04928 <sup>b</sup>
Body weight gain (gm)	93.25±18.84 <sup>a</sup>	576.0±23.25 <sup>d</sup>	461.0±28.18 <sup>c</sup>	328.6±31.89 <sup>b</sup>
FCR	4.269±1.096 <sup>a</sup>	1.766±0.07514 <sup>b</sup>	2.223±0.2494 <sup>ab</sup>	2.009±0.3049 <sup>b</sup>
Feed-intake (gm/day)	47.57±3.003 <sup>a</sup>	111.3±3.522 <sup>b</sup>	131.0±2.964 <sup>c</sup>	91.60±3.778 <sup>d</sup>
Water consumption (ml/day)	298.2±11.39 <sup>a</sup>	393.2±5.770 <sup>b</sup>	317.4±2.606 <sup>a</sup>	313.9±5.837 <sup>a</sup>
Performance index (%)	109.5	145.9	131.6	129.6
Mortality rate (%)	55	11.2	33	44
Economic efficiency (%)	27.6	42.4	36.7	33.5

\*Means ± Standard error which superscripts with different small letters (a-c) within the same row differ significantly at  $P < 0.05$ .

Concerning to some reproductive traits of WNZ rabbits exposed to sever heat stress under the warm subtropical environmental conditions of Egypt, group (I) showed that there was a significant decrease ( $P < 0.05$ ) at conception rate, gestation period, litter size at birth and weaning, litter weight at birth and weaning, and estimated milk yield comparing with treated groups. The gestation period seemed to be affected by heat stress, as the gestation period was decreased (29 days) in rabbits exposed to high ambient temperature (control group) comparing with treated ones (30 days). The number of kits was found to be lower in the control group (4 kits) than those treated by honey supplementation (8 kits), drinking cool water (7kits) and injection of Levamisole (6 kits) as shown in table 4. The results are in a harmony with those of Marai et al. (2002, 2004, 2006); Balabel (2004) and Abdel-Monem et al. (2013) who declared that an elevation of ambient temperature had a negative impact on appetite and accordingly on feed intake that ends with slowing growth and impairment of reproduction in rabbits. Moreover, these results may be attributed to the decrease in fertility and conception rate under high environmental temperature as a complex set of events were expressed in a significant reduction in total young born and in an increase in percentage of young born dead (Matassino et al., 1970). Where all the above-mentioned findings of decreased reproductive traits for untreated stressed group may emphasize the hypothesis of El-Masry et al. (1994) which approved that conception rate could be decreased under heat stress condition due to decline in live sperm concentration with a significant alteration in the levels of seminal plasma composition. Such decrease in kit's weight at birth and weaning at untreated stressed group may be attributed to, hypothermic condition of pregnant dams that leads to decrease feed intake, depressed thyroid activity and hence, metabolic rate resulting in decrease in the litter weight at birth, in addition such dams had low milk yield resulting in less feed for the growing young. Among treated groups honey treated group still represented as the much better group in most of reproductive traits conception rate (88), fetal losses (zero), dead kits before weaning (1.4±0.44), Litter weight at birth (270.3±15.33 gm.), Kits body weight at weaning (396.2±2.916 gm), Kits weight gain (576.0±23.25 gm) and milk yield (135.6±3.735 gm/day), followed by treated group by drinking cool water and finally the group injected by Levamisole. It should be noted here that honey administration possesses better feed utilization especially starch and mineral (El Nagar et al., 2010). The honey action mechanism could partially explain the increased pre-weaning weight gain, milk yield, as well as the heavier weaning weights and lower preweaning deaths.

Elevated temperature significantly affected the measured hematological parameters as shown at tables 5, which showed significant ( $P < 0.05$ ) decreases in RBC, PCV, Hb, WBC and neutrophils at untreated group comparing with treated groups. On the other hand, significant ( $P < 0.05$ ) increases were found in numbers of lymphocytes in the heat stressed rabbits compared to the treated groups. Basophiles were unaffected by elevated temperature. These results are in agreement with the findings of Ondruska et al. (2011), who reported that heat stress in mammals decreased the level of ACTH, which might then result in decreases in RBC counts, PCV, and Hb concentration. In addition, the depression of PCV during the hot season was also reported to be related to a reduction in cellular oxygen, a requirement for reducing

metabolic heat production in order to compensate for the elevated environmental heat load (Okab and El-Banna, 2003 and Okab et al., 2008).

**Table 4.** Reproductive traits of White New Zealand rabbits under Egypt summer conditions, 19 June- 21 August, 2015.

Examined items	Examined groups			
	Control group	Honey treated group	Cool water treated group	Levamisole injected group
Conception rate %	44	88	44	44
G. P (days)	29.67±0.2108	30.56±0.2940	30.75±0.2500	30.80±0.3742
Milk yield (gm/day)	93.33±8.999 <sup>a</sup>	135.6±3.735 <sup>b</sup>	131.8±4.765 <sup>b</sup>	100.8±6.538 <sup>a</sup>
Litter size	4.833±0.4773 <sup>a</sup>	8.222±0.3643 <sup>b</sup>	7.750±0.4532 <sup>b</sup>	6.000±0.3162 <sup>a</sup>
Fetal losses	0.3333±0.237	-	-	0.3333±0.167
Dead kits before weaning	1.556±0.5031	1.444±0.4444	1.556±0.4444	1.333±0.4714
Litter weight at birth (gm)	185.7±14.58 <sup>a</sup>	270.3±15.33 <sup>b</sup>	258.1±15.32 <sup>b</sup>	230.2±11.79 <sup>ab</sup>
Kits body weight at weaning (gm)	269.5±7.390 <sup>a</sup>	396.2±2.916 <sup>b</sup>	391.6±2.748 <sup>b</sup>	345.4±15.77 <sup>c</sup>
Kits weight gain (gm)	93.25±18.84 <sup>a</sup>	576.0±23.25 <sup>b</sup>	461.0±28.18 <sup>b</sup>	328.6±31.89 <sup>c</sup>

\*Means ± Standard error which superscripts with different small letters (a-c) within the same row differ significantly at P<0.05.

**Table 5.** Respiratory rate, rectal temperature, select hematological and biochemical parameters measured in the blood of White New Zealand rabbits under Egypt summer conditions, 19 June-21 August, 2015.

Examined items	Examined groups			
	Control group	Honey treated group	Cool water treated group	Levamisole injected group
<b>Hematological Parameters</b>				
RBCs (x10 <sup>6</sup> /μL)	4.033±0.2404 <sup>a</sup>	7.633±0.6438 <sup>b</sup>	6.267±0.2603 <sup>b</sup>	7.133±0.2333 <sup>b</sup>
Hb (g/dl)	7.933±0.2028 <sup>a</sup>	13.23±0.2728 <sup>b</sup>	10.17±0.2028 <sup>c</sup>	13.40±0.5508 <sup>b</sup>
PCV (%)	28.00±1.528 <sup>a</sup>	38.67±0.8819 <sup>b</sup>	35.33±1.856 <sup>b</sup>	40.33±0.8819 <sup>b</sup>
WBCs (μL)	3833±145.3 <sup>a</sup>	9533±409.6 <sup>b</sup>	6733±145.3 <sup>c</sup>	10567±688.8 <sup>b</sup>
Neutrophils (%)	35.33±6.741 <sup>a</sup>	53.33±1.453 <sup>b</sup>	51.67±3.283 <sup>ab</sup>	58.33±2.028 <sup>b</sup>
Lymphocytes (%)	59.67±6.960 <sup>a</sup>	40.33±0.8819 <sup>ab</sup>	41.67±4.978 <sup>ab</sup>	31.67±1.764 <sup>b</sup>
Basophils (%)	4.667±0.3333	4.333±0.3333	4.333±1.202	6.667±0.3333
<b>Serum Biochemical Analysis</b>				
Glucose (mg/dl)	47.67±3.383 <sup>a</sup>	178.0±2.517 <sup>d</sup>	93.33±3.480 <sup>b</sup>	131.0±6.083 <sup>c</sup>
Total protein (g/dl)	4.767±0.1453 <sup>a</sup>	6.567±0.2333 <sup>b</sup>	4.833±0.1453 <sup>a</sup>	5.833±0.2186 <sup>b</sup>
Albumin (g/dl)	2.567±0.2186 <sup>a</sup>	4.500±0.2082 <sup>b</sup>	2.467±0.0882 <sup>a</sup>	3.767±0.3528 <sup>b</sup>
Globulin (mg/dl)	2.200±0.3512	2.067±0.4410	2.367±0.067	2.067±0.5239
A/G ratio	1.281±0.3595	2.421±0.5816	1.042±0.0251	2.250±0.8539
Urea (mg/dl)	59.33±4.910 <sup>a</sup>	33.67±4.096 <sup>b</sup>	18.67±1.453 <sup>b</sup>	27.67±3.283 <sup>b</sup>
Creatine (mg/dl)	2.900±0.1155 <sup>a</sup>	1.367±0.3383 <sup>b</sup>	0.4000±0.058 <sup>b</sup>	1.167±0.3180 <sup>b</sup>
<b>Respiratory Rate</b>				
(breath/minute)	180.5±0.6118 <sup>d</sup>	154.5±1.156 <sup>b</sup>	150.6±0.9445 <sup>a</sup>	169.0±0.6213 <sup>c</sup>
Rectal temperature (°C)	40.00±0.02295 <sup>a</sup>	39.57±0.03500 <sup>b</sup>	39.44±0.05179 <sup>c</sup>	39.97±0.02054 <sup>a</sup>

\*Means which superscript with different small letters (a-c) within the same row differ significantly at P<0.05; RBCs: Red Blood Cells; Hb: Haemoglobin; PCV: Packed Cell Volume; WBCs: White Blood Cells; A/G ratio: Albumin Globulin ratio.

Serum biochemical analysis of the examined groups as shown at Table 5, showed significant (P<0.05) decreases of serum blood glucose (mg/dl), total protein (g/dl), albumin (g/dl), globulin (mg/dl) and albumin globulin ratio at untreated stressed group in comparing with treated groups. On other hand there was a significant (P<0.05) increase of serum urea (mg/dl) and creatine (mg/dl) level among the untreated group comparing with treated groups. The results are in a harmony of those given by Habeeb et al. (1993) and Marai et al. (1999) who reported that albumin was significantly lower when the animals were exposed to heat stress conditions. Also Habeeb et al. (1997) showed that blood glucose was decreased significantly in NZW rabbits exposed to heat stress conditions by 20.7%. The decrease in plasma glucose could also be due to the marked dilution of blood and body fluids as a whole. As well as, the increase in glucose utilization to produce more energy for greater muscular expenditure required for high respiratory activity (Habeeb et al., 1993). Moreover Okab et al. (2008) declared that decrease in glucose levels in the heat stressed adult rabbits could be due to increases in glucose utilization during muscular movements required for high respiratory activity or due to increases in corticosteroid concentrations (Habeeb et al., 1997). On the same context honey treated group showed the same trend of significant P<0.05 increase in the level of serum glucose, total protein, albumin, globulin and A/G ratio. The results are in agreement of Elnagar et al. (2010). Also, similar to the findings of Kurkure et al. (2000) who reported

increased serum albumin when white Leghorn cockerels were orally given 10 ml/bird/day RJ. On other hand the group treated with cool drinking water showed the much better records for decreased level of serum urea and creatine level. This indicates an improved kidney function in cleansing blood especially with the elevated blood proteins observed in this study (Elnagar et al., 2010).

Data presented in table 5, show significant differences ( $P < 0.05$ ) in rectal temperature ( $^{\circ}\text{C}$ ) and respiration rate in the examined groups where it recorded higher records at untreated stressed group comparing with treated groups. The results are in coincide with Marai et al. (2001) and Marai et al. (2007) who reported the highly significant increase in thermoregulatory parameters (respiration and temperatures of ear, rectum and skin) due to exposure of the animals to severe heat stress .It is well known that, adult rabbits are homoeothermic and are provided with physiological mechanisms by which they can maintain their deep body temperature constant within the thermo neutral zone. The increase in rectal temperature of the heat stressed rabbits may be due to failure of the physiological mechanism (Marai et al., 2001).The increase in respiration frequency and evaporative water loss is linearly related to the increase in ambient temperature above the panting threshold (Richards, 1976). Thus enables the animals to dissipate heat by vaporizing high moisture through the respiratory air, which accounts to about 30% of total heat dissipation. Respiration becomes the main pathway for loss of the latent heat, since most sweat glands in rabbits are not functional and perspiration is not great. Among the treated groups, cool water drinking group was much better group in improving rectal temperature and respiratory rate followed by honey treated group. The results are in coincide with Marai et al. (1999) who reported that drinking cool water acts through cooling the animal's body core by conduction as a result to the difference between temperatures of the drinking water and urine, mediated by cooling the area of the hypothalamus. Together with the high specific heat of water as well as, body water retention with drinking water that help to alleviate the rise in body temperature which are reflected in reduction of rectal temperature and respiration rate.

## CONCLUSION

From these results, it could be concluded that the adverse impact of exposure of growing rabbits to severe heat stress under the warm subtropical environmental conditions of Egypt could be mitigated through addition of honey to drinking water also via drinking cool water. This could minimize reproductive losses, as they have positive effect on rabbit's reproductive traits via increase conception rate, litter weight at birth, kits body weight at weaning and weight gain and milk yield. On other hand, decrease fetal losses .Furthermore, improve most of rabbit's performance traits.

## Acknowledgements

The authors are highly thankful to the all staff members of the Department of Hygiene and Preventive Medicine, Faculty of Veterinary Medicine Kafrelsheikh University, Egypt for their kind help and assistance for carrying out this research work. This study was not funded by any institute and authors used their own fund.

## Competing interests

The authors have no competing interests to declare.

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## Dog Bites and Rabies: A Decade Perspective in Nigeria (2005-2014)

Tekki Sini Ishaya\*, Odita Christiana Ibrinke, Idachaba Ejura Stella, Akanbi Babatunde Olatunde, Moses Davou Gyang, Barde Joshua Israel, James Ahmed Saidu, Rimfa Amos Gambo, Kumbish Rinle Peterside, Agama Christianah, Zhakom Ponfa Nden and Okewole Ademola Philip

Central Diagnostic Laboratory, National Veterinary Research Institute, P.M.B 01, Vom, Nigeria

\*Corresponding author's email: ishaya.sinitekki@yahoo.com

### ABSTRACT

Rabies is a fatal zoonotic encephalitis caused by the rabies virus commonly transmitted to human and other mammals by dog bites. A 10 year review of dog bite cases in humans from 2005 to 2014 was undertaken from archives of the rabies laboratory, National Veterinary Research Institute (NVRI) Vom, Nigeria to assess the magnitude of dog bites and associated risks of human exposure to rabies among bite victims. Of the 1, 840 cases reported, the highest and the lowest rates of bite occurred on 2009 and 2007 respectively. Children constituted 31.5% of the victims, 36.0% were adults, while 32.5% had no age indications. Male victims formed 46.7% of the cases, 38.4% involved females while the genders of the remaining 14.9% were not given. Similarly, prevalence of rabies cases were highest and lowest in 2009 and 2007 respectively while the overall prevalence of rabies-positive dog bite cases during the decade were high (61.1%). However, rabies public campaigns by indigenous veterinary professional groups during the initial editions of the world rabies day improved the level of awareness, which possibly led to the rise in reported cases of dog bites in 2009, while the considerable drop in the cases and probably in rabies in subsequent years, could have been due to vaccination of a considerable number of the dog population. Appreciable reduction in dog bite cases and in rabies nationwide in Nigeria, are only achievable when stakeholders determine to tackle dog bites by supporting responsible ownership and annual mass vaccination of dogs and cats against rabies as well as quarantining or controlling their movements. In rural Africa, where the risk of dog bites and rabies is greatest, it is important to raise public awareness on the roles of accurate laboratory diagnosis and surveillance in the national rabies control and monitoring program.

**Key words:** Dog bite cases, Humans, Rabies, Nigeria

### INTRODUCTION

Rabies is caused by a negative sensed, ssRNA-virus in the Lyssavirus genus, Rhabdoviridae family of the Mononegavirales order and results in approximately 59,000 human deaths yearly worldwide (Freuling et al., 2012 and Hampson et al., 2015). It is characterized by gross neglect and under reporting (Mallewa et al., 2007) in regions where it is endemic due to ignorance of its dangers, human exposure, poverty, traditional beliefs and limited political will on the part of the political authorities (Ezebuoro et al., 1980 and Tekki et al., 2013). Consequently, victims of infection may not receive appropriate medication and could die unreported (Hampson et al., 2015). Rabies is mainly transmitted to humans through bites by infected animals, especially dogs, cats and bats, via their infective saliva (Christopher and Pereira, 1972; Kasempimolporn et al., 2000; WHO, 2007). Significant number of dog bites occurred in tens of millions of people worldwide each year (WHO, 2013), mostly in children (Ellis and Ellis, 2014; Presutti, 2001 and WHO, 2013). In Nigeria, approximately 99% of human rabies resulted from bites of domestic dog, which is the major maintenance and vector host species of the virus causing the disease with wildcats, jackal species, hyena and civets as possible wildlife reservoir hosts (WHO, 2013). Karshima et al. (2013) reported an incidence of 46.7% rabid dog bites among humans in a review of two months cases of rabies in a government veterinary clinic while Aworh et al. (2011) reported a prevalence of 52.9% of dog bites in children in 10 years study carried out in a hospital in Nigeria. However, the magnitude of dog bites and their consequences in rabies transmission from a nationwide perspective is not known. Therefore, the study was designed to determine the prevalence of dog bites and investigate the associated risks of human exposure to rabies among the bite victims, from the analysis of available data on confirmatory diagnosis of rabies at the National Veterinary Research Institute (NVRI), Vom, Nigeria from 2005 to 2014.

### MATERIALS AND METHODS

The National Veterinary Research Institute, Vom, Nigeria, has as one of its mandates, the diagnosis of animal diseases of economic and public health importance, including rabies, in the country. Animal specimens were received from all over the country for diagnosis of various animal diseases in the facility. For post mortem confirmatory diagnosis

ORIGINAL ARTICLE  
pii: S232245681600003-6  
Received: 18 Jan 2016  
Accepted: 17 Feb 2016

of rabies in animals, specimens submitted to the laboratory included: the head or fresh brain, salivary gland or at times a whole carcass of rabid suspect animal in cold condition or in 50% glycerol saline. Dog bite cases, ages and genders of dog bite victims and rabies infection status of the dogs involved in the bites between 2005 and 2014 were reviewed and results presented by descriptive statistics. During the period under review, a total of 2141 dog samples were received and tested for rabies.

## RESULTS

The overall prevalence of dog bite cases was 85.9% (1840/2141), while the overall prevalence of dog bite cases that tested positive was 61.1% (1124/1840). Of the total 1840 dog bite cases among humans, the highest was 16.2% (298/1840 cases) which occurred in 2009, followed by 13.4% (246/1840 cases) in 2011 while the lowest of 5.9% (109/1840 cases) was recorded in 2007, followed by 7.1% (130/1840 cases) in 2010 (Figures 1 and 2). The highest prevalence (85.2%) of bite cases by rabid dogs occurred in 2009 followed by 74.6% in 2010 and 73.0% in 2008, while the lowest prevalence of 42.2% followed by 44.0%, were recorded in 2007 and 2006 (Figure 3) respectively. However, the overall prevalence of rabies-positive dog bite cases during period under review was 61.1%.

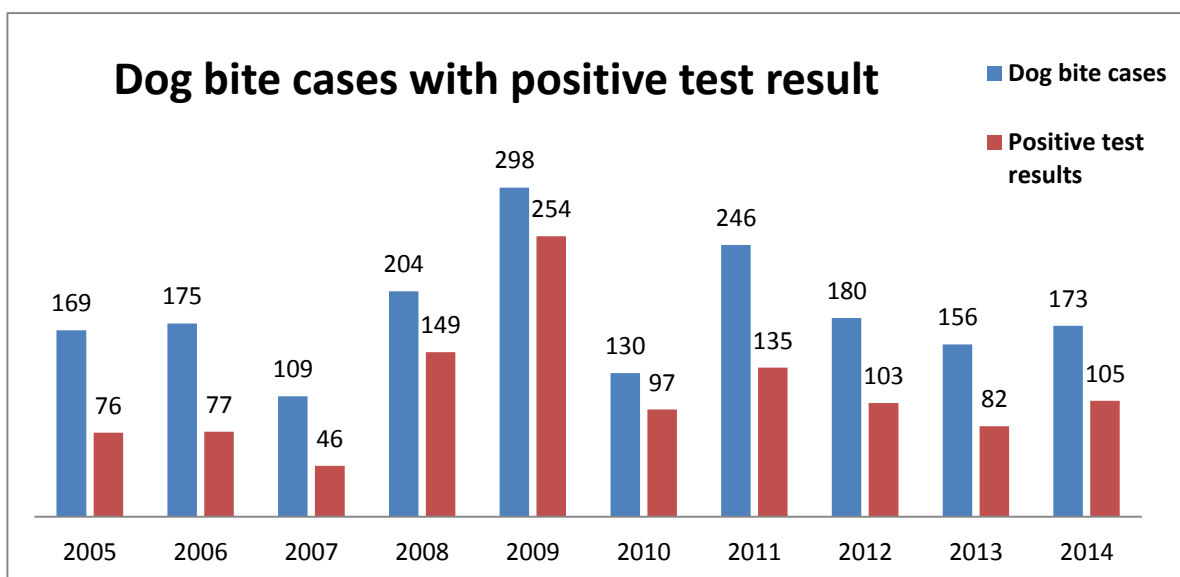
Total bite victims that were children were 579 (31.5%) of which 333 (57.5%) were males, 222 (38.3%) were females but the ages and genders of 24 (4.2%) were not on record (Figure 4). A total of 663 (36.0%) adults were bitten, with 335 (50.5%) males, 316 (47.7%) females while the genders of 12 (1.8%) adults were not provided. However, the ages of 598 (32.5%) of the victims were not on record. In addition, 860 (46.7%) of the cases documented involved male victims, 707 (38.4%) involved females while the genders of 273 (14.9%) of the victims were not supplied to the laboratory (Figure 5). Record of vaccination of the dogs involved in the bite cases were as follows: dogs with up to date vaccination were 45 (2.4%), 29 (1.6%) had expired vaccination, 944 (51.3%) had no history of vaccination and 822 (44.7%) had no information on vaccination (Figure 6).

## DISCUSSION

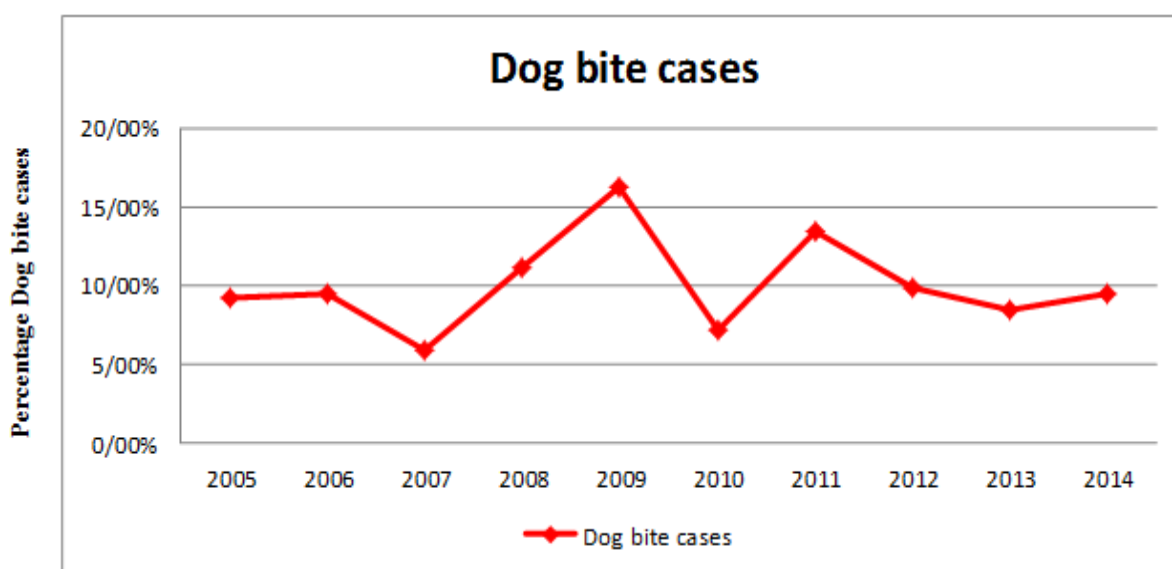
Eleven dog bite cases per million and 6.6 positive cases per million people (using population estimate of 170 million), and the mean annual dog bite cases of 184 in Nigeria as recorded in the study was quite alarming. Although the mean bite cases was much higher than the 92 dog bite cases reported from a similar study conducted in 20 local government areas of Bauchi state in the country the true figure of dog bites among humans in the country was certainly much higher as the only cases received at the rabies diagnostic facility were represented in this study (Bello et al., 2007). Many other cases must have occurred unreported as previously documented (Fagbami et al., 1981 and Mallewa et al., 2007). Overall prevalence of rabies-positive dog bite cases in this study was slightly higher than the incidence of 46.7% rabid dog bites in humans in a review of two months cases of rabies in a government veterinary clinic in Nigeria (Karshima et al., 2013). As expected, the national prevalence of dog bite cases based on sample submission to the diagnostic facility over the years was higher than in the localized hospital/clinic studies (Karshima et al., 2013). In this study, there was an approximately two fold increase in prevalence dog bite cases from 2007 to 2008 and from 2010 to 2011, while there was more than two fold drop in prevalence from 2009 when the dog bite cases were highest, as compared to 2010 (Figure 1). The rise in prevalence of dog bites in 2009 (Figure 2) could be an indication of improvements in reporting dog bite cases following supposed increase in awareness on rabies through public lectures, talks, radio and television programs and free dog rabies vaccination campaigns observed during the 2008 and 2009 editions of the WRD by various groups of veterinarians, paravets and veterinary students across Nigeria (Tekki et al., 2013). Much of these campaigns were carried out, in the central states of Nigeria, from where majority of diagnostic specimens were received. It was however observed that the prevalence of dog bite cases and perhaps rabies cases, dropped considerably in subsequent years (Figure 2) probably due to a good number of dog population getting vaccinated during the above mentioned campaigns (Tekki et al., 2013).

An increase in the prevalence of bite by dogs that tested positive for rabies was also observed from 2008 to 2010 (Figure 3). This increase was ascribable to the replacement of the less sensitive "Sellers staining diagnostic technique" previously employed in rabies diagnosis, with a more sensitive, specific and globally acceptable fluorescent antibody test in 2009, which could have led to achievement of accurate diagnosis, in addition to the previously mentioned possible causes (OIE, 2013). Although, there were relative drops in prevalence of rabies among biting dogs from 2011 and above (Figure 3), these were still higher (above 50%) than it were between 2005 and 2007 (below 50%), due probably to the previously mentioned campaigns and adoption of the more sensitive diagnostic technique. Also, although the 31.3% involvement of children in dog bite cases observed in this study (Figure 4) was quite high, it disagreed with the 52.9% reported in another study carried out in a hospital in Nigeria (Aworh et al., 2011). However, this rate of dog bites was slightly lower than the 63% reported in Iraq by Horton et al. (2013). It is not unlikely that under reporting as well as poor

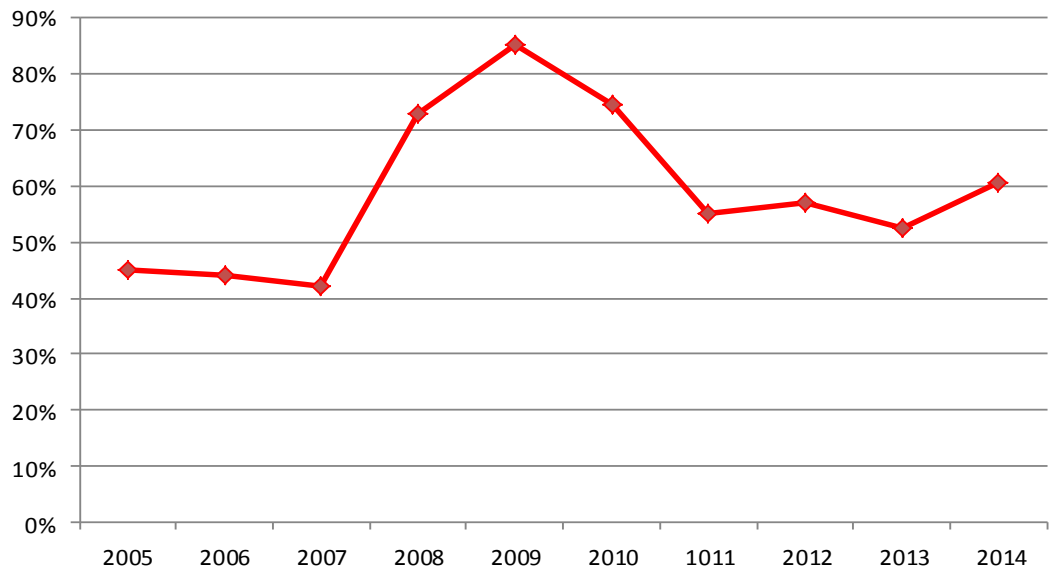
documentation of cases from the field is responsible for this disparity in incidences. Non-availability of records of age groups of children in which 32.5% of the cases occurred (Figure 4) and the genders of bite victims in 14.9% of cases (Figure 5) were evidences of poor and under reporting of cases in this study. Contrary to popular reports of higher incidence of dog bites in children than adults (Familusi and Moore, 1972; Presutti, 2001 and WHO, 2013), we observed a higher occurrence in adults in this study. Majority of dog bites in children have been reported to be due to provocation of dog by chasing/teasing, pulling its tail, fur or ear, playing with the dog when sick, when eating or with its food (Buchanan, 2015). The dog may also be overprotective while the child is ignorant of boundaries and limits (Buchanan, 2015). Due to their body size in relation to little children, dogs may consider they are superior and would not dread the children. In addition people, especially Africans, do not educate their children on how to handle and act around animals (WebMD, 2015). The highest risk of rabies is in the poorest regions of the developing countries, where domestic dog vaccination against rabies is not extensively executed, coupled with limited access to post exposure prophylaxis (Hampson et al., 2015). Neighbourhood or community dogs and outright stray dogs are poorly fed and not confined, but gather in packs and facilitates easy contact between dogs and humans/domestic animals/wildlife and cycle of dog bites and endemic canine rabies can be easily maintained with increased human exposure risk to rabies (Atuman et al., 2014). These have resulted in approximately 59,000 annual premature death and loss of productivity due to rabies (Hampson et al., 2015).



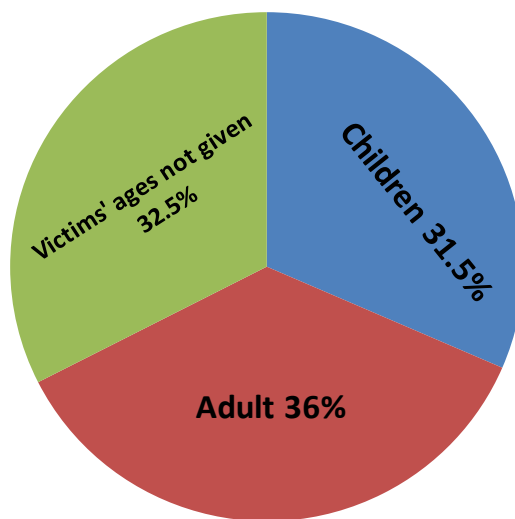
**Figure 1.** Annual human dog bite cases in Nigeria from 2005 to 2014, showing numbers of confirmed rabies positive cases



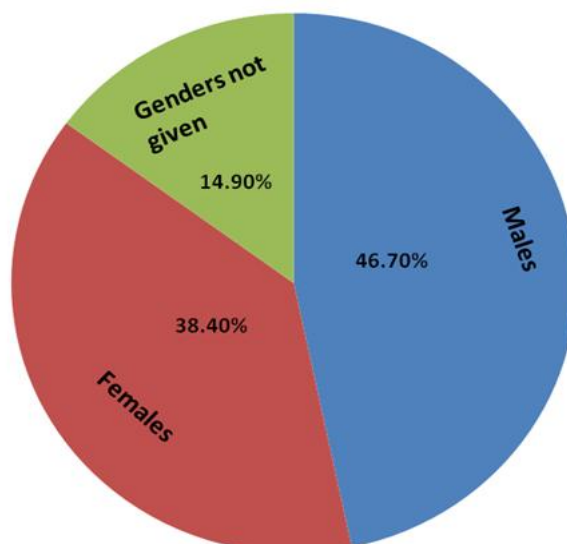
**Figure 2.** Percentage of annual human dog bite cases in Nigeria, from 2005 to 2014



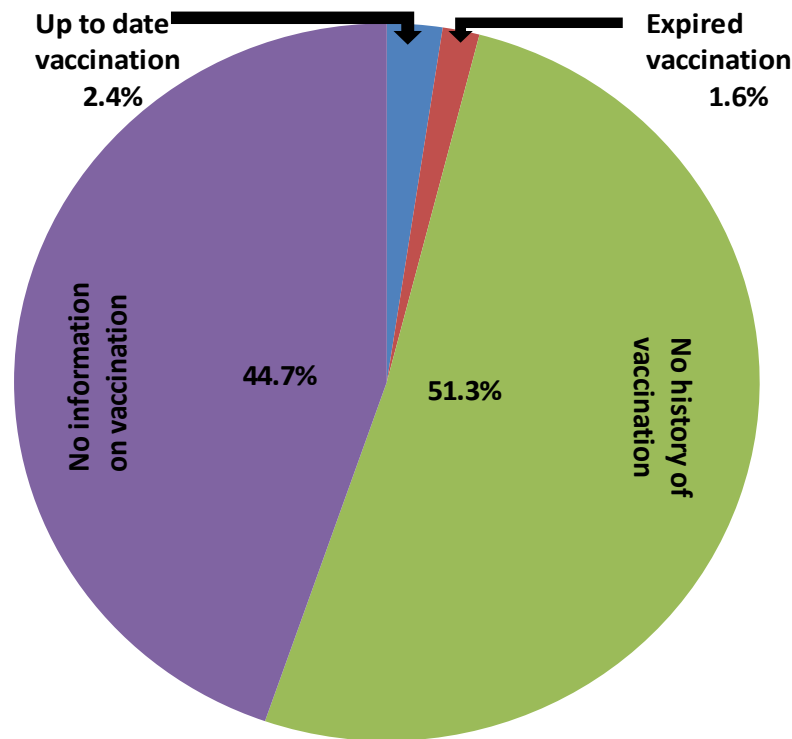
**Figure 3.** Percentage of annual dog bites cases confirmed to be rabies positive in Nigeria from 2005 to 2014



**Figure 4.** Overall distribution of dog bite cases by age of victims in Nigeria, from 2005 to 2014



**Figure 5.** Overall distribution of dog bite cases by genders of victims in Nigeria, from 2005 to 2014



**Figure 6.** Overall distribution of dog bite cases by rabies vaccination status of biting dogs in Nigeria, from 2005 to 2014

## CONCLUSION

Dog bite cases recorded in the last decade in Nigeria were quite alarming. The overall prevalence of rabies-positive dog bite cases during the period was also very high. Proofs of under reporting of cases as previously reported were observed in the study. For an appreciable reduction in dog bite cases and rabies in affected regions therefore, all tiers of government, NGOs, public spirited organizations and individuals need to make concerted efforts in tackling dog bites and rabies by embarking on or supporting annual vaccination of dogs, cats, foxes and sylvatic reservoir hosts against rabies as well as quarantining or controlling their movements. Creation of public awareness in rural Africa, where the risk of dog bites and rabies is greatest, are also necessary measures in reducing the risks. Accurate laboratory diagnosis and surveillance need to be supported by all stakeholders for effective control program.

### Acknowledgements

The authors thanked the management of the National Veterinary Research Institute, Vom for creating an enabling environment for this work to be carried out.

### Competing interests

The authors have no competing interests to declare.

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## A Review Article of Artificial Insemination in Poultry

**Tarekegn Getachew**

*School of Animal and Range Sciences, Haramaya University, P.O.B: 138, Haramaya, Ethiopia.*

Corresponding author's Email: taregech23@gmail.com.

### ABSTRACT

The objective of this review is to discuss the history, importance of Artificial Insemination (AI), semen collection and deposition techniques, physiology of cockerel reproduction, characteristics and chemical components of chicken semen, semen storage, and evaluation of semen, semen extenders and behavior of sperm in the oviduct. The first AI in poultry was reported in 1936. All of the avian male reproductive system is inside the bird unlike the males of mammalian species. Avian semen contains energy source to help the viability of semen for AI. For the purpose of in vitro storage semen extenders with appropriate osmolarity and source of energy can be used. Abdominal massage method is the most common technique to collect semen. The technique involves restraining the male and gently stroking the back of the bird from behind the wings towards the tail with firm rapid strokes and gentle squeeze to extract the semen. There are two methods of semen deposition in poultry. These methods are the Intra-peritoneal insemination and vaginal insemination. Vaginal insemination is the commonly used method. It involves everting (turning inside out) the cloaca to expose the reproductive tract of the female.

**Key words:** Artificial insemination, Extenders, Ova, Semen

### INTRODUCTION

Assisted Reproduction Technologies (ART's), such as Artificial Insemination (AI) contribute to increase poultry production, as it allows a wider use of genetically superior cockerels with a high productive performance. The development of AI technique has allowed the rapid dissemination of genetic material from a small number of superior sires to a large number of females (Vishwanath and Shannon, 1997). The impacts of AI on genetic improvement and control of venereal diseases has been the greatest (Foote, 2002). Poultry producers over the years have used genetic selection and improved nutritional management practices as a result there has been a steady and rapid increase of the growth rate in poultry production which in turn has certain detrimental effects on reproduction (Bramwell, 2002).

Due to the sharp increase in chicken meat consumption it has also become important to increase the production of layers to meet the demand. Assisted reproduction technologies (ART's), such as AI contribute to increase poultry production, as it allows a wider use of genetically superior cockerels with a high productive performance (Benoff et al., 1981). On the other hand, ART's have the potential benefit of allowing the preservation of semen collected from these cockerels for future use and for export if necessary.

Artificial insemination has been considered as a valuable technique in the poultry industry (Benoff et al., 1981). One of the advantages of this technology over natural mating is the efficient use of males. This in turn, decreases the cost of AI directly by reducing the number of cocks needed (Benoff et al., 1981). When fertility in the broiler breeds continues to decline due to the fact that males are selected for growth coupled with compatibility problems between large and smaller breeds, AI may become effective in broiler breeder management and in solving compatibility problems (Reddy, 1995). In addition to its breeding significance, AI is important in controlling venereal diseases. Birds present special challenges in disease control (Blanco and Hofle, 2004).

#### **Artificial insemination in poultry**

AI in poultry was first successful in 1899 when Ivanov produced fertile chicken eggs using semen recovered from the ductus deferens after killing a cock (Lunak, 2010). The most widely used technique of intravaginal insemination was first reported by Quinn and Burrows in 1936. The avian male reproductive system is all inside the bird unlike the males of mammalian species which have their reproductive systems outside of the body cavity (Brooks, 1990). This is one of the really remarkable things about birds where the sperm remain viable at body temperature. Mammalian sperm does not remain viable at body temperature which is the reason the male reproductive organs are found on the outside of the body (Brooks, 1990).

According to Aisha and Zain (2010) AI in poultry is the process by which semen from male bird is collected and then introduced to females for the purpose of fertilizing eggs. The main objectives of AI in poultry are to place the

REVIEW ARTICLE  
pii: S232245681600004-6  
Received: 07 Feb 2016  
Accepted: 16 Feb 2016



required dose of semen into the oviduct of the female so that it is deposited near the sperm storage glands and to carry out the AI process with due regard to the best health and welfare of the breeder females thereby, achieving the highest fertility levels possible. During insemination, the volume of semen required is generally less than 0.1 ml, within a minimum of  $100$  to  $200 \times 10^6$  viable sperm per insemination within the hen's vagina (Gordon, 2005).

Biologically, after deposition of semen in the oviduct the semen will enter the sperm storage gland, situated at the junction of the vagina and the shell gland and from here the spermatozoa will make their way up the oviduct to a second storage site situated at the junction of the magnum and infundibulum (Aisha and Zain, 2010). The passage of an ovum into the infundibulum stimulates spermatozoa activity and fertilization of the ovum by one sperm takes place (Aisha and Zain, 2010).

### **Controlling diseases**

Birds present special challenges in disease control (Blanco and Hofle, 2004). The semen diluents may be another common source of contamination, especially for *E. coli* and *pseudomonas* (Van Eck and Goren, 1980). These agents can trigger significant sperm mortality in raw or diluted ejaculate and when used for AI may cause both systemic disease and infertility (Van Eck and Goren, 1980). This problem is commonly addressed by adding antibiotic to diluents (i.e., penicillin, Gentamicin and streptomycin) although these drugs may adversely impact on sperm viability (Donoghue et al., 2004).

Collectively, results to date strongly emphasize the need to minimize ejaculate contamination by focusing on sanitary semen collection and processing (including using appropriate and prudent doses of broad spectrum antibiotic and antifungal) as well as protecting birds from pathogens. This includes maintaining thorough pathogen monitoring protocols and even strict isolation/ quarantine practices for breeder populations (Turin et al., 1999). Once infectious situations arise, rapid mitigation is mandatory, although it is challenging to alleviate certain viruses (i.e., West Nile virus) from infected birds (Turin et al., 1999).

### **Poultry semen collection techniques**

In 1937 Burrows and Quinn described a non-invasive method, the abdominal massage method for collection of semen from roosters. The technique involves restraining the male and gently stroking the back of the bird from behind the wings towards the tail with firm rapid strokes. The male responds with tumescence erection of the phallus, at which time the handler gently squeezes the cloaca extracting semen through the external papillae of the ductus deferens (vas deferens) collecting the semen into a container.

As stated by Burrows and Quinn (1937), the techniques of AI actually begin prior to the procedure. It includes housing the male poultry away from the hens maximizes the amount of available semen. Because the bird's phallus is located in the same duct as his anus, removing food 12 hours prior to collection will help prevent fecal contamination of the semen. Roosters and tom turkeys need to be routinely primed for semen collection for several days prior to the actual AI procedure to guarantee that each bird is fertile with a microscopic examination of the sperm. According to Burrows and Quinn (1937), as with semen collection of other farm animals, one must stimulate the bird's sexual organ to extend outside of his body. One person can handle this procedure with small birds such as chickens or quail; it normally takes two people with a large turkey or a goose.

### **Physiology of cockerel reproduction**

Sperm Production is initiated by adequate secretion of Gonadotropin Releasing Hormone (GnRH) from the hypothalamus, the secretion of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) by the anterior lobe of the pituitary and the secretion of the gonadal steroids (testosterone and estrogen). LH acts on the Leydig cells within the testes to stimulate the production of progesterone, which is converted to the male sex hormone testosterone (Senger, 2003). Testosterone within the seminiferous tubules is essential for spermatogenesis, while the Leydig cells become unresponsive sustaining high levels of LH (Senger, 2003).

The testes are surrounded by a layer of connective tissue containing the seminiferous tubules and Leydig cells. Several androgens are produced in the interstitial cells of the testes, but the major hormone in the blood, is testosterone. Testosterone is essential for the development of the secondary sex characteristics and for normal mating behavior in the males. It is also necessary for the functioning of the accessory glands, sperm production and the maintenance of the male duct system. This hormone also aids in spermatocytogenesis, the transport of sperm and deposition of sperm in the female reproductive tract (Beardon et al., 2004). As the cockerel reaches maturity, the production of testosterone is stimulated by the increasing concentration of circulating gonadotropins (Etches, 1996).

The major gonadotropins involved are FSH and LH, which are also called the Interstitial Cell Stimulating Hormone (ICSH) in males. Both of the gonadotrophic hormones are secreted by the anterior pituitary (Salisbury et al., 1978). FSH as such, acts on the germinal cells in the seminiferous tubules of the testes and supports spermatogenesis to the

secondary spermatocytes stage. LH stimulates the Leydig cells to produce testosterone and other androgens (Hafez and Hafez, 2000).

### Spermatogenesis

Spermatogenesis is the process of division and differentiation by which sperm are produced in the seminiferous tubules of the testes and consists of two phases, namely spermatocytogenesis and spermiogenesis (Gordon, 2005). According to the previous author, spermiogenesis is a metamorphic process in which no cell division is involved and a string of events result in the formation of the sperm tail. Alteration in the sperm morphology can be seen in the nuclear proteins, cellular size, cellular shape and the position of the acrosomal granules and localization of the centrioles. The number of sperm produced is dependent on the number of Sertoli cells and Leydig cells present. The Golgi apparatus is one of the cell organelles, located near the sperm nucleus and which give rise to the subcellular organelle known as the acrosome. The acrosome develops and forms a cap over the anterior portion of the nucleus and spreads until it covers two-thirds of the anterior nucleus (Senger, 2003). During the maturation phase, the spermatids are completely differentiated with the final formation of the flagella (principal and endpiece), assembly of mitochondria (midpiece), the neck piece and complete condensation and shaping of the nucleus (Beardon et al., 2004).

### Factors affecting semen production

There are inherent variations in semen production between different species of poultry and between individuals within strains and breeds (Lake, 1983). Other than in the mammals, cockerel sperm is generally immotile before ejaculation (Hafez and Hafez, 2000). According to Anderson (2001) there are many factors that may influence the production of semen and a thorough knowledge of the physiology of cockerel reproduction is essential to enable an understanding of male fertility. There are also many external and internal factors that may affect the male and may influence the production of semen. The reproductive functions in the male are endocrine controlled by the pituitary, testes and to a certain extent external factors.

The certain external factors affecting reproductive efficiency in the cockerel can be grouped into two categories firstly, the direct influence of the diet, management, and the normal physiological processes that regulate the activities of spermatogenesis and secondly, factor that influence the degree to which the male will respond to the massage technique during semen collection (Maule, 1962).

### Characteristics and chemical components of chicken semen

In the male, semen is composed of sperm and seminal plasma secreted by the epididymis and vas deferens. The sperm are produced in the testes, and in the case of the avian species the seminal fluid is also produced in the testes. All these secretions in the testes are controlled by the endocrine hormones carried to them in the blood stream. The pituitary FSH and LH regulates the testes, which in turn produce testosterone, which controls the testicular development and secretions (Hafez, 1974).

**Table1.** Characteristics and the mean chemical components of semen in cockerel

Characteristics and Components	Cockerel
Ejaculate volume (ml)	0.2-0.5
Sperm concentration ( $\times 10^6$ /ml)	3000-7000
Sperm/ejaculate ( $\times 10^9$ )	0.06-3.5
Motile sperm (%)	60-80
Morphologically normal sperm (%)	85-90
Protein (g/100 ml)	2.8
pH	7.2-7.6
Fructose (mg/100ml)	4
Sorbitol (mg/100ml)	0-10
Inositol (mg/100ml)	16-20
Glycerolphosphoryl choline (GPC) (mg/100ml)	0-40
Ergothioneine (mg/100ml)	0-2
Sodium (mg/100ml)	352
Potassium (mg/100ml)	61
Calcium (mg/100ml)	10
Magnesium (mg/100ml)	14
Chloride (mg/100ml)	147

### **Gross evaluation of semen**

Determination of the viability of spermatozoa after semen storage is important for several reasons. First, it provides an estimate of semen quality. If inseminated with poor quality semen, it reduces fertility, increases embryo mortality and forces the hen to rely on spermatozoa from previous inseminations (Thurston, 1995). More traditional semen evaluation procedures include determination of semen volume, color, concentration, motility, viability and morphology of spermatozoa. Many of these assessments correlate with the fertilizing capacity of spermatozoa when fresh semen is evaluated (Wishart, 1995). Histological and fluorescent stains have been used to determine live/dead sperm ratios and metabolic activity (Lake and Stewart, 1978a; Lake and Stewart 1978b and Chaudhuri et al., 1988).

### **Semen color**

The color of semen is generally an indicator of the density of the ejaculate. The semen of the domestic fowl varies from a dense opaque suspension to a watery fluid secreted by various reproductive glands. It ranges from a relative high sperm density or degrees of clear to milky white, with declining sperm numbers (Peters et al., 2008). The color of semen may depend on the species of bird used, but generally semen should be creamy which indicates a high sperm concentration (Cole and Cupps, 1977). Color could also serve as an indicator of contamination by e.g. feces or urine and thus become brown or green in color (Lake, 1983). Sometimes flakes of blood may be present, which may be a result of excessive force during the collection process or injury. Semen samples that are contaminated by feces do not have to be discarded, but diluted with antibiotics e.g. penicillin and dihydrostreptomycin or neomycin to reduce the loss of sperm. This however is not recommended. Antibiotics can also increase fertility when used as a diluent in semen (Bearden et al., 2004).

### **Ejaculate volume**

The cockerel produces between 0.1 ml and 1.5 ml per ejaculation, with 0.6 ml being the average ejaculate volume recorded (Cole and Cupps, 1977). Different cockerels of the same species often produce different volumes of semen at different times (Anderson, 2001).

The average volume ejaculated using the abdominal massage technique is approximately 0.25ml (Gordon, 2005). Bah et al. (2001) found the mean semen volume to be  $0.28 \pm 0.14$ ml. However, the recorded semen volume was found to range between  $0.37 \pm 0.02$  and  $0.73 \pm 0.01$  ml (Peters et al., 2008). It is important to realize that semen volume and sperm concentration (volume multiplied by the concentration) will determine the total number of sperm collected per ejaculation. This could facilitate the determination of the number of insemination doses that can be prepared (Senger, 2003).

### **Semen pH**

The semen pH varies slightly between different breeds and bird species. The optimum semen pH ranges between 7.0 and 7.4. Sperm motility is generally high between a pH of 7.0 and 7.4 (slightly alkaline) and also increases the fertilizing ability, compared to a pH of 6.4 (acidic), which is not suitable for semen preservation, as it may cause damage to the plasma membrane of the sperm cell (Latif et al., 2005). Contrary, Donoghue and Wishart (2000) reported several trials that indicate that chicken sperm can tolerate a pH range of 6.0 to 8.0. Peters et al.(2008) also found the semen pH of the cockerel to be slightly alkaline, with a mean of  $7.01 \pm 0.01$ , while Bah et al.(2001) recorded a semen pH ranging between  $7.54 \pm 0.04$  to  $7.80 \pm 0.03$ .

This variation in semen pH may be due to many factors. The pH, especially that of ejaculated semen is dependent on several secretions involved. Poor quality semen generally contains large amounts of fluid from the accessory glands, which increases the semen pH (Salisbury et al., 1978). The pH of semen is likely to decrease as the time between collection and measurement increases, and the semen collection tubes are narrow in shape causing sperm to break down fructose in the semen to lactic acid under anaerobic conditions. Semen samples that contain many dead sperm may evolve to ammonia, which will also increase the pH (Salisbury et al., 1978).

### **Microscopic evaluation of cockerel semen**

#### **Motility**

Sperm motility assessment is indicative of the viability of sperm and the quality of the semen sample. Evaluation of sperm motility is conducted with fresh and extended semen, and generally analyzed under the light microscope (10× magnifications) (Hafez and Hafez, 2000). Evaluation of raw semen gives the performance of the sperm in its own accessory gland fluid, which is often hindered when higher sperm concentrations make it difficult to distinguish individual sperm motility patterns (Table 2). Hence an aliquot of semen is usually extended prior to evaluation (Hafez and Hafez, 2000).

**Table 2.** Motility patterns of sperm from sub fertile or infertile cockerels

Pattern of sperm motility	Sperm tail	Sperm head	Sperm movements and progression
Vibratory circular	Slow or rapid quivering from side to side, vibrations of various types and frequency bent in curved shape, immotile	Immotile or vibrating in one place	Motility without progression, perpendicular, oblique or horizontal clockwise or counterclockwise motion
Darting	Vibration with high velocity	Irregular, propelling, no rotation	Minimal and erratic, wandering path
Rotating	Undulations of small amplitude pass down tail	Whole sperm rotates around its axis, periodic flashing effect	Rapid forward progress in a straight line
Asymmetric head and /or flagella	Amplitude of tail wave is asymmetric at both sides	Irregular, propelling, usually no rotation	Circular orbits if rotational motile is absent

### Sperm morphology

Normally the sperm cell consists of a head, midpiece and tail portion. The head contains the nucleus, containing the genetic material, which is the sire's genetic contribution to the offspring (Tuncer et al., 2006). The post-nuclear cap which covers the posterior part of the nucleus and acrosome which covers the anterior part of the nucleus both protect the nucleus. If the acrosome is malformed or damaged the sperm cell will not be able to fertilize the ova by penetrating the zona pellucida. Acrosome, sperm head, middle piece and tail deformations in fresh white leghorn cockerel ejaculates have been recorded to be  $0.62 \pm 0.04\%$ ,  $1.34 \pm 0.05\%$ ,  $2.47 \pm 0.05\%$  and  $2.89 \pm 0.08\%$  respectively (Tuncer et al., 2006). While Tuncer et al., (2008) also recorded values of acrosome, sperm head, middle piece and tail deformations  $0.39 \pm 0.03\%$ ,  $1.06 \pm 0.03\%$ ,  $2.32 \pm 0.05\%$  and  $2.53 \pm 0.04\%$  respectively in cockerels.

Blesbois (2007) described an eosin-nigrosin stain technique to assess the morphology of cockerel semen. Semen was mixed with 1.6% eosin and 6% nigrosin, diluted in 20 $\mu$ m Beltsville Poultry Semen Extender (BPSE), diluted semen in 2ml stain solution and incubated for 2 minutes before being spread on a microscope slide. The stain is dried and observed under a light microscope (1000 $\times$  magnification). Sperm morphology can serve as an indicator of semen quality and shortcomings in the male. The success of this evaluation technique depends on how the stain was prepared and used, while other more advanced laboratories use a computer analysis system for sperm evaluation. Eosin-nigrosin is a dye commonly used in laboratories to determine abnormalities and smears are made, immersed in oil and observed under the light microscope. Viable, non-viable, properly formed, live, and damaged sperm can be determined using this evaluation (Lukaszewicz et al., 2008).

The sperm morphology of poultry semen differs from that of mammals. However a difference also exists between domestic birds, even though the shape and size of the sperm cell are similar. In poultry the sperm cell is surrounded by the cytoplasmic membrane and the acrosome has an inner spine surrounded by a conical shaped cap. The head of the sperm contains the nuclear material of the gamete, while the midpiece consists of the cylindrical centrioles surrounded by a sheath of mitochondria (Hafez, 1974). The midpiece of cockerel sperm is considerably longer, compared to other species, approximately one quarter longer and this property makes poultry sperm to have more midpiece bending than other species. According to Alkanet et al., (2001) the in vitro assessment morphological sperm defects of cockerel semen include; neck bending (mid piece bending), mid piece damage, acrosome damage (bending, swelling, knotting or rounding), total head swelling and tail defects.

### Semen concentration

Gordon (2005) stated semen collected from domestic cockerel contains an average sperm concentration of  $5000 \times 10^6$  sperm/ml. On the other hand the report of Hafez and Hafez (2000) stated that semen collected from domestic cockerel contains an average sperm concentration of  $3000-7000 \times 10^6$  sperm/ml.

### Factors affecting semen quality (motility, morphology and viability)

The assessment of semen quality characteristics of poultry birds gives an excellent indicator of their reproductive potential and has been reported to be a major determinant of fertility and subsequent hatchability of eggs (Peters et al., 2004). According to Hafez (1978), the differences in volumes and sperm concentration of the domestic fowl semen depends largely on the relative contribution of the various reproductive glands, the number of spermatozoa that could be obtained from a breed/strain and the extent to which the genetic potentials can be exploited.

Breed and seasonal differences in semen production of cocks was reported by Saeed and Al-Soudi (1975) while Egbunike and Oluyemi (1979) showed that breed and time of semen collection affects cock semen. Omeje and Marine (1990) observed that significant genotype differences affected body size and semen characteristics of cocks, except the pH value. In addition, age by genotype interaction effect was important only for semen volume.

Only morphologically normal spermatozoa are capable of ascending through the vagina of the hen to the region where the sperm storage tubules are located (Bakst et al., 1994). Sperm motility is a primary determinant of fertility in domestic fowl (Donoghue et al., 1998). Sperm mobility is a function of the product of motile concentration and the

proportion of motile sperm with a straight line velocity >30 m/s (Froman et al., 2003). The hormone level in seminal plasma is a direct reflection of male testicular endocrine activity. The relationships between semen quality and concentration of testosterone in avian seminal plasma have been discussed (Zeman et al., 1986). High quality semen determined by the condition of normal spermatogenesis. Estrogen, in addition to testosterone plays a role in the development and function of the testis and male reproductive tract (Rivas et al., 2002 and Akingbemi, 2005).

### **Semen extenders**

The use of AI in poultry can be enhanced with the improvement of diluents and method of storing semen (Mian et al., 1990). The advantage of semen dilution includes the maximum use of good quality semen in short supply; reduction in the ratio of males to female and valuable sires with low semen quantity can be used for many females. On the contrary, it is difficult to handle the desired very small volume of undiluted semen and expel it from a tube because of its viscous nature. However, diluents make it possible thereby enabling the spread of semen over many more hens (Mian et al., 1990).

Diluents are buffered salt solutions used to extend semen, they maintain the viability of spermatozoa in vitro, and maximize the number of hens that can be inseminated. Semen diluents are based on the biochemical composition of chicken and turkey semen (Lake, 1995). Addition of various components to semen maintains motility, fertilizing capacity and preserve sperm membrane integrity (Riha et al., 2006 and Sarlos et al., 2002). Glutamic acid, the most prominent anionic constituent of avian seminal plasma, became a standard component of diluents (Lake and Mc Indoe, 1959). Egg yolk is generally accepted to be an effective agent in semen extenders for protection of spermatozoa against cold shock and the lipid phase transition effect (Aboagla and Terada, 2004). However, the use of chilled stored semen diluted in egg yolk based semen extenders is limited by its relatively short time fertilization capacity and individual differences in egg yolk due to different period of egg storage (Aurichet et al., 1997).

Both hypertonic and hypotonic extenders reduce the metabolic activity of the sperm, and could disrupt the cell membrane integrity that leads to the clumping of the sperm (Latif et al., 2005). Glutamic acid is the most prominent anionic constituent in avian seminal plasma, and is a standard component of all semen diluents. Basic characteristics common to all diluents include the maintenance of pH, osmolarity and the provider of energy for the sperm. The motility and metabolic rate of sperm can thus be altered by decreasing the diluent below pH 6.0. So for example a low pH reduces the sperm motility and a high pH increases the metabolic rate in vitro (Donoghue and Wishart, 2000). Modified Ringer's solution with the following composition of sodium chloride (68g), potassium chloride (17.33g), calcium chloride (6.42g), magnesium sulphate (2.50g), sodium bicarbonate (24.50g) and distilled water, can be used to dilute poultry semen (Martin, 2004).

According to Jones and Mann (1973) and Jones et al. (1979) spermatozoa is extremely sensitive to oxidative damage. Lipid peroxidation plays a leading role in aging of spermatozoa, shortening its life span in vitro and affecting the preservation of semen for AI. The process of peroxidation induces structural alterations; particularly in the acrosomal region of the sperm cell, a fast and irreversible loss of motility, a deep change in metabolism and a high rate of release of intracellular components. Lipid peroxidation has been defined as an important aspect of oxidative stress in mammalian spermatozoa for many years (Jones et al., 1979).

### **Semen osmotic pressure in poultry**

Latif et al., (2005) concluded that an increase in the osmotic pressure can be ascribed to the contamination of broiler semen with urine and bacteria, which in turn results in the clumping of sperm. A 375mOsm/kg osmotic pressure is optimum for the short term storage of semen. However the recommended osmolarity of the Blom stain technique is lower and was quantified as 220 mOsmol/kg dissolved in diluents, with composition similar to that of seminal plasma. However these hypo osmotic conditions resulted in the swelling of the sperm head (Lukaszewicz et al., 2008). The semen diluents must be isotonic, as the osmotic pressure created by the solution may be detrimental to the sperm cell (Senger, 2003).

### **Short term poultry semen preservation**

Semen diluents are currently being used for both short and long term storage of domestic fowl semen. These extenders are being commercialized to improve the general reproductive effectiveness of the cockerels and lower the cost of AI. The development of semen diluents initially began with the use of NaCl (saline) solutions. Now complex diluents containing different osmotic regulators, energy sources and buffers are being used (Bootwalla and Miles, 1992). The most common practice for short term fowl semen storage (hours to days at a temperature of -4°C) requires the suspension of sperm in a suitable extender to maintain the sperm viability, in vitro. Assessment of diluted and undiluted stored cockerel semen revealed that the application of extenders is essential to sustain sperm quality (Bootwalla and Miles, 1992).

It was established that diluted fowl semen could be stored for up to 24 hours, without impairing the viability and fertilizing capacity of the sperm (Siudzinska and Lukaszewicz, 2008). Several other factors play a role in sustaining the quality of semen during storage over time e.g. the diluents used in semen extension and storage conditions e.g. time, aeration and storage temperatures. It is known that sperm motility and the fertilizing capacity of undiluted raw fowl semen stored in vitro usually decreases within 1h after collection (Dumpala et al., 2006). Therefore, to store cockerel semen, the type of diluents and storage temperature is very crucial.

Generally an extender will facilitate semen handling procedures, particularly during collection and evaluation, by maintaining the sperm viability, but preventing their activation. For semen maintained at 41°C and diluted BPSE or Minimum Essential Medium (MEM) there were quadratic and linear increases in the percentage dead sperm over time, while a drastic linear increase existed for undiluted semen. There was thus a linear decrease in Sperm Quality Index (SQI) for undiluted and semen diluted in MEM over time (4°C). However, for semen diluted with only BPSE, there was a linear increase in SQI (Dumpala et al., 2006). Extenders serve to also protect the sperm cells from chemical and physical changes and contamination in their environment and provide more favorable conditions for fertilization (Chulhong and Chapman, 2005).

### **Poultry AI technique**

Generally there are two methods of semen deposition in poultry. These methods are the intra peritoneal insemination and vaginal insemination. The most reliable and successful routine for insemination of poultry, is by depositing semen directly in the mid vaginal area (Cole and Cupps, 1977).

### **Intra peritoneal insemination**

This technique of AI is not reliable and has been used periodically for many years. In this technique a sharp needle is punched through the abdominal wall and the cannula inserted to deposit semen in the region of the ovary (Cole and Cupps, 1977).

### **Vaginal insemination**

This is the most commonly used AI procedure and two personnel are required for this operation. The technique was developed in the 1930s and involves applying pressure to the hen's abdomen and everting (Turn inside out) the vaginal orifice through the cloaca (Quinn and Burrows, 1936; Cole and Cupps, 1977). This procedure is also referred to as cracking, venting or everting the hen. Semen is deposited 2–4 cm into the vaginal orifice concurrently with the release of pressure on the hen's abdomen. Insemination is accomplished with sterile straws, syringes or plastic tubes. In large scale commercial operations, automated semen dispensers using individual straws loaded with a set AI dose are commonly used.

Because poultry semen loses viability within 1 hour, hen insemination should begin immediately after collection (Aisha and Zain, 2010). You begin by holding the hen upside down against your body in the same way you held the rooster or tom. Exerting firm pressure on the left side of the vent causes the cloaca (the urogenital opening in birds) to evert, you need to use your thumb and forefinger to expose the oviduct (vagina). According to the same author (2011), the oviduct is the opening on the left side of the cloaca next to the anus. You can insert the insemination tube as far as possible up the oviduct, then squeeze out the semen and release the pressure on the cloaca at the same time. Relaxing the cloaca draws the semen further into the hen's body. Chicken inseminations can be completed within two consecutive days and then once a week after that. Because most hens will carry an egg in their oviducts in the morning, thus obstructing the route of the sperm to the ovary, insemination should occur in the afternoons after laying (Aisha and Zain, 2010).

### **Behavior of sperm in the oviduct of the hen**

Froman and Feltmann (2005) reported that the hen's Sperm Storage Tube (SST) is located between the vagina and shell gland of the oviduct. Previously sperm residing in the SST were considered to be immotile; however it is likely that storage depends on moving against a generated by the SST epithelial cells. Cockerel sperm are motile at a body temperature of 41°C for an interval of days to weeks following ejaculation. How the sperm enter, survive, and exit these SST however is not known. Movement of sperm to the uterovaginal region is fast, however only viable sperm enter the SST. Current evidence suggests that the release of stored sperm is episodic, although it was first thought to be associated with oviposition.

Movement of sperm through the oviduct is achieved by smooth muscle contractions and/or ciliary activity and accumulates in the mucosal folds and short tubular glands at the lower end of the infundibulum (Hafez and Hafez, 2000). According to Hafez and Hafez (2000) the sperm in mammals spend a relatively short time in the female tract, while in chickens and the turkey sperm can spend a much longer period of time in the oviduct before fertilizing the egg yolk cell, up to 32 days in the chicken and 70 days in the turkey. Tabatabaei et al. (2009) stated that although the process of

prolonged sperm storage is not known, it is thought to include a reversible suppression of respiration and motility of the sperm, as well as stabilization of the plasma membrane and maintenance of the acrosome.

According to Mauldin (2000), sperm are released from the SST to fertilize the sequentially ovulated ova at regular intervals. After release the sperm are taken to the ovum by contraction of the hen's oviduct, and sperm motility is no longer critical. Within 5 to 10 minutes after ovulation, sperm has already moved to the genital disc on the surface of the ovum. The sperm that make contact with the perivitelline layer of the ovum undergo an acrosome reaction and, presumably by the action of the trypsin-like enzyme acrosin, hydrolyze the perivitelline layer. Theoretically only one sperm fertilizes the ovum, but polyspermy has been observed in the hen ovum with many holes hydrolyzed in the perivitelline (Hafez and Hafez, 2000).

### Competing interests

The authors have no competing interests to declare.

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## Intestinal Ulceration in West African Mud Turtle (*Pelusios Castaneus*)

Olayinka-Adefemi Folayemi<sup>1\*</sup>, Abdulraheem Zainab Omolara<sup>1</sup>, Olatunji-Akioye Adenike<sup>1</sup>, Ogbole Inalegwu Godwin<sup>2</sup> and Olukole Samuel Gbadebo<sup>3</sup>

<sup>1</sup>Faculty of Veterinary Medicine, University of Ibadan, Nigeria

<sup>2</sup>College of Medicine, University of Ibadan, Nigeria

<sup>3</sup>Faculty of Veterinary Medicine, University of Ibadan, Nigeria

\*Corresponding author's email: folayemiadefemi@gmail.com

### ABSTRACT

An adult female West African mud turtle (*Pelusios castaneus*) that had been acquired as part of study on the digestive anatomy on the *P. castaneus* presented mild signs of anorexia that had persisted for a week. On radiographic examination of the digestive tract using Barium sulphate contrast agent, a normal study was observed but an area of contrast coating remained at the region of the duodenum following excretion of the contrast agent. The digestive tract was isolated and gross examination of the coated area revealed areas of echymotic hemorrhage and ulcers in this turtle. With not much scientific research available on this wildlife species, this case of a gastrointestinal tract abnormality is probably the first report of a digestive tract pathology seen in this tropical fresh water turtle.

**Key words:** *Pelusios castaneus*, Duodenum, Echymotic hemorrhage, Ulcer

CASE REPORT  
pii: S232245681600005-6  
Received: 13 Jan 2016  
Accepted: 21 Feb 2016

### INTRODUCTION

The West African mud turtle, *Pelusios castaneus* is a small sized turtle of the family Pelomedusidae (Broadley, 1973; TTWG, 2014) known to inhabit the fresh waters of the West African river banks (Kirkpatrick, 1995; Broadley and Boycott, 2009). This turtle is a pleurodiran turtle which retract its head into the carapace by bending its neck to the side hence is called West African side-neck turtle or simply African side-neck turtle (Omonana et al., 2011; Olukole et al., 2014b). This turtle is known to have a massive population boom during the raining season and the International Union for the Conservation of Nature Red List of Threatened Species (IUCN, 2015) has classified them as Least Concern (LN).

Despite this classification, the *P. castaneus* still faces threats of diseases, illegal transportation and threats as well massive killings of the specie in south-western Nigeria for fetish practices (Olukole et al., 2014a). Of recent studies are being done to understand the peculiarities of the *P. castaneus*. Omonana et al. (2011) studied their blood profile to give an insight into their hematology and Olukole et al. (2014a, 2014b and 2014c) has studied their carapace, the reproductive anatomy of the male *P. castaneus* as well as the vertebral formula of this Tropical turtle.

Gastrointestinal tract pathologies have been major accidental findings in large sea turtles. A case of intestinal obstruction in the Loggerhead sea turtle, *Caretta caretta* was reported by Di Bello et al. (2006) while Oros et al. (2005) attributed an integral cause of mortality among sea turtles in the canary islands to gastrointestinal tract disease in which most of these discoveries of perforations and obstructions from monofilament lines and fishing hooks were made on post-mortem. This finding of intestinal tract pathology in the *P. castaneus* was accidentally discovered during a study designed to understand the Digestive anatomy of this specie of turtle.

### CASE REPORT

An adult female West African side-necked turtle was recently acquired as part of a research study group. The ethical approval for the study was obtained from the Animal Care and Use Research Ethics Committee, University of Ibadan and reference number assigned as UI-ACUREC/App/2015/041.

The turtle was derived from nature with no discernable malformation. It weighed 1.5kg and was housed in a box cage with a mate. It fed on commercial feed pellets formulated for turtles and was allowed daily swim in a shallow pool. It presented with mild anorexia that was observed to have last for seven days.

On physical examination of the turtle which was carried out by examining the skin for dermatologic lesions, observing the carapace and plastron for signs of cracks as well as the gait for signs of orthopedic abnormalities, it was concluded that the turtle physically appeared to be in good health.

Contrast radiography (Dorso-ventral view) done using 10ml Barium Sulphate and the Allegers® model digital X-ray equipment at radiographic setting 80kv and 200mAs for 0.3seconds (Santos et al, 2010). Dorso-ventral view was

preferred over lateral view to avoid superimposition of the coelomic structures (Valente et al., 2007). The serial radiographs were taken for day 0, 24 hours and 48 hourly till day 16 when the digestive tract was isolated for gross study.

The turtle was placed on deep sedation using Ketamin HCL at a dosage of 25mg/kg intramuscularly via the thigh muscle after which cervical decapitation was done (Olukole et al., 2014a and 2014b). The plastron was separated from the carapace and then the digestive tract was separated for gross examination and laboratory analysis (Wyneken, 2001). A swab of the tract and a fine needle biopsy of the affected portions were taken for culture and cytology respectively.

## RESULTS

Administration of the contrast reviewed a radio-opaque outline of the tract and the turtle excreted the contrast 48hours after administration. Following excretion of the agent, there remained a radio-opaque portion of retained contrast coating in the intestine (Figure 1). This region of coating was localized to the proximal portion of the intestine. Gross examination of the tract revealed this region was the proximal one-third of the duodenum. This intestinal mucosa in this region was ulcerated and had ecchymotic hemorrhages. The swab results showed solely the presence of the normal intestinal micro flora and on cytology the mild presence of inflammatory cells and duodenal mucosa cells.

## DISCUSSION

Digestive tract abnormalities are the most common disorders often identified in turtles (Valente et al., 2007). This is because most often the causes are usually man-made activities that encroach on the habitat and life patterns. These man-made activities include habitat disruption, pollution of their habitat with harmful objects like fishing hooks and line and the extreme conditions they face during illegal transportation and trade (Maran et al., 2002; Oros et al., 2005). Gastro intestinal tract abnormalities have been reported in the sea turtles where they were discovered either by diagnostic imaging or as accidental findings on post-mortem examinations (Di Bello et al., 2006).

There are few reports on the West African mud turtle (*Pelusios castaneus*) and of these none described the digestive tract in this specie. Apart from mild anorexia, the turtle described in this study showed no clinical signs despite this pathology. This lack of clinical signs and symptoms is generally expected in turtles that have also been described as exothermic and hardy. It is for this reason that even the most skilled exotic pet veterinarian turn to additional diagnostic tools like imaging for definitive diagnosis (Banzato et al., 2013).

The serial radiographs of this turtle hinted of an intestinal lesion that was confirmed on post-mortem to be duodenal ulcers (Figure 1 and Figure 2).



**Figure 1.** Radiograph showing contrast coating of the duodenum 48hours after contrast excretion in the *Pelusios castaneus*



**Figure 2.** Ulcerated duodenum and ecchymotic hemorrhage on day 16 after contrast excretion in the *Pelusios castaneus*

Reports show that ulcers generally have different etiopathologies. They can result from the imbalance of digestive fluids; they could be triggered by stress, bacterial infection (*Helicobacter pylori*) and neoplasia (gastrinomas) (Najm et al., 2011 and Milosavljenic et al., 2011). In turtles, foreign body ingestion is another etiology (Oros et al., 2005 and Valente et al., 2007). This case of an ulcer observation in this turtle is an accidental discovery and the diagnostic methods employed to investigate the cause of this lesion yielded no specific result. No foreign object was identified in the tract on post-mortem and no obstruction or tissue growths were observed. Furthermore the swab and biopsy taken of the lesion yielded no incriminating pathogenic agent.

This ulcer most possibly was triggered by stress factors such as capturing, handling and transporting on acquisition or the recent change in habitat of the turtle from the riverbanks to the veterinary facility. In conclusion, this finding may also be case of an idiopathic ulcer in this West African mud turtle.

## CONCLUSION

The fact that this discovery was made accidentally further presses on the authors opinion that diagnostic imaging should be done as routine checks for turtles and reptiles when they are presented at veterinary centers whenever there is suspicion of disease or if they are presented in apparently 'good health'. This would be most useful not only in diseases diagnosis and therapy but also as a prophylactic measure to maintain health and institute prompt treatment when accidental findings are discovered via radiography. To the authors' knowledge, this is the first report of intestinal ulceration in the West African mud turtle and there are no conflicts of interest regarding the publication of this paper.

### Acknowledgement

The authors would like to acknowledge the members and the staff of Two Tees Diagnostic Centre for the use of their facility in carrying out this study.

### Competing interests

The authors have no competing interests to declare.

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**ISSN:** 2322-4568

**Frequency:** [Quarterly](#)

**Current Volume:** 6 (2016)

**Publisher:** [Scienceline Publication](#)

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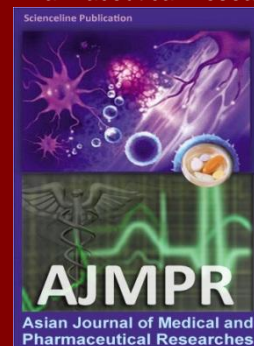
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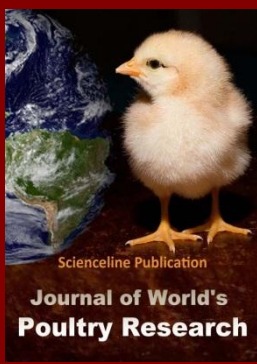
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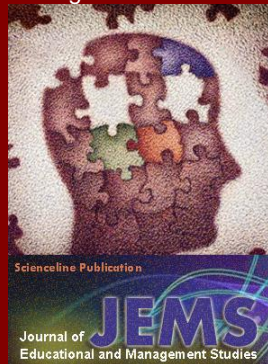
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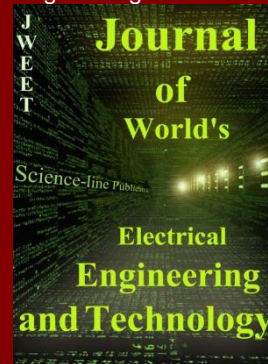
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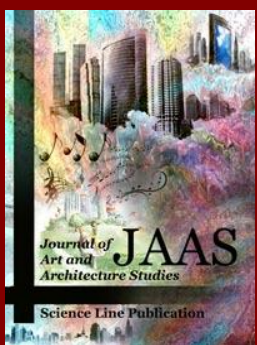
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