Biochemical effects of *Trichomonas Gallinae* on pigeons reared on irrigation channels water and water treated with anti protozoal agents

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A B S T R A C T

This study was carried out on 50 healthy squabs; up to 3 week of age. Squabs were housed in clean separate metal cages. They were kept at a constant environmental and nutritional condition throughout the period of experiment. The birds were left 5 days for acclimatization before the beginning of the experiment. The main objective of this study was to determine the effectiveness of the anti-protozoal agent devices (Sodium hypochlorite pump – UV. disinfection unite – Ozone gas generator) in water treatment station in inactivating Trichomonads protozoan that survive in water via determination the Biochemical changes of serum Blood hemoglobin concentrations (gm/dl), Liver function tests (ALT, AST, GGT, ALP and Bilirubin), Lipid profile including (Total Cholesterol and TG), Brain function tests (MAO, ChE, 5́-NT and CK), Serum immunoglobulins (IgA, IgG and IgM) and Serum proinflammatory cytokines (IL-2 and IL-6). The results of the present study showed that, infected squabs with trichomoniasis suffered from anemia, impaired liver and brain function, impaired lipid metabolism. Chronic inflammatory state evidenced by significant elevation in IL-2, IL-6 and IgM.

Keywords: water treatment, ultraviolet radiation, Ozone gas, Chlorination

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1. INTRODUCTION

Water is essential to sustain life, and a satisfactory adequate, safe and accessible supply must be available to all (WHO, 2004). Water has long served as a mode of transmission of diseases. The most important waterborne diseases are those of the intestinal tract, including typhoid fever, paratyphoid, dysentery, infectious hepatitis, cholera, and some parasitic worm diseases, (Wisner and Adams, 2002). The protozoan Trichomonas gallinae, is a cosmopolitan parasite of pigeons and doves which may be transmitted from one bird to another by contaminated drinking water with feces, saliva or crop secretions, (Kocan, 1969). Trichomonas gallinae, the etiologic agent of avian trichomonosis, is a flagellated protozoal parasite in the class Zoomastigophorea and order Trichomonadida. The protozoa are found in the upper digestive tract of clinically and subclinically infected birds. Trichomonosis generally manifests as a caseous, ulcerative mass within the oral cavity. Disinfection process is the process of eliminating pathogenic organisms in water and is achieved by their inactivation in the water or their physical removal from the water. Disinfection technologies can be broadly categorized as chemical- and physical-based systems (U.S.E.P.A., 2004). Chlorine is the most widely utilized disinfectant in water treatment, (Yen, 2007). Water treatment projects often utilize sodium hypochlorite (NaOCl) as a chlorine source. NaOCl disassociates in water to Hypochlorite (HOCl). HOCl is cost effective, easy to manage and efficient at disinfecting a variety of pathogens (March et al., 2005). Ultraviolet (UV) irradiation is an electromagnetic wave having a wavelength of 100 – 400 nanometers (nm), which is between the X-ray and visible ray

spectrums (U.S. E.P.A., 1999). Ozone is a strong oxidant and potent disinfectant (Weiner, 2008).

2. MATERIAL AND METHODS

2.1 Pigeons:

This study was carried out on 50 healthy squabs; up to 3 week of age. Squabs were housed in clean separate metal cages. Squabs were kept at a constant environmental and nutritional condition throughout the period of experiment.

2.2 Parasite:

The diagnosis of trichomoniasis was confirmed by observing Trichomonas gallinae on wet-mount preparations of samples collected from lesions and caseated mass in the crop of diseased pigeons using moistened microbiology swabs, according to Samour and Naldo (2003). Samples were examined by high power for detection of Trichomonas gallinae. Permanent slides were stained with Giemsa stain and examined under oil immersion lens (Levine, 1985).

2.3 Culture Techniques:

Wet swabs were prepared, by gentle rotation, through the lesions. The swaps were dipped into culture-media Glucose-serum broth media (G.S.B) according to Nagwa (1995). The inoculated tubes were incubated at 37°C for 7 days. One drop, from the bottom of each tube, was microscopically examined 4 and 7 day post-inoculation (PI) for the presence of motile trichomonads according to Abd El-Motelib and Galal (1993).

2.4 Chemicals and Units:

Sodium hypochlorite (1 mg/l), chemix company Egypt. Aquafine® 60" 254 nm UV disinfection lamps, a trojan technologies company USA. ozonia ozone generator unit (0.1 mg/l), ozonia company Switzerland.

2.5 In vitro studies

In vitro trials were made in order to treat trichomonads in water source. These treatments are chlorine in the form of aqueous hypochlorite solution Sodium hypochlorite, Ultraviolet radiation, and Ozone gas as follow:

- Ten tubes of G.S.B.M. containing motile trichomonads were inoculated in one liter distilled water (non-treated water)
- Ten tubes of G.S.B.M. Containing motile trichomonads were inoculated in one liter distilled water and treated with Sodium hypochlorite (1 mg/L); (Chlorine treated water).
- Ten tubes of G.S.B.M. containing motile trichomonads were inoculated in one liter distilled water and treated with Aquafine® 60" 254 nm UV disinfection lamp for 10 minutes (UV. Treated water).
- Ten tubes of G.S.B.M. containing motile trichomonads were inoculated in one liter distilled water and treated with ozone gas (0.1 mg/L) ;(Ozone treated water).

2.6 In vivo studies:

Squabs were divided into five groups, each group contains squabs as follow: Group 1: Negative control group, contained 10 squabs drinking distilled water. Group 2: Positive control group, contained 10 squabs drinking non-treated water. Group 3: Chlorine treated group, contained 10 squabs drinking Chlorine treated water. Group 4: UV. Treated group, contained 10 squabs drinking UV treated water. Group 5: Ozone treated group, contained 10 squabs drinking Ozone treated water.

2.7 Blood samples:

Blood samples were collected after overnight fasting from all groups after two weeks. Two blood samples were collected from wing veins of squabs in each group. The first sample was collected on EDTA for hematological examination .The other sample was collected on clean sterile tube for serum and separated by centrifugation at

3500 r.p.m for 20 minutes after clotting of the blood. The following parameters were determined: Blood hemoglobin concentrations (gm/dl). Liver function tests (ALT, AST, GGT, ALP and Bilirubin). Lipid profile including total Cholesterol and TG. Brain function tests (MAO, ChE, 5́-NT and CK). Serum immunoglobulins (IgA, IgG and IgM). Serum proinflammatory cytokines (IL-2 and IL-6).

3. RESULTS

2.1. Blood hemoglobin concentrations

Squabs of positive control group exhibited significant decrease in HB. Concentration compared to negative control group. But squabs of Chlorine treated group, UV treated group and Ozone treated group exhibited anon significant decrease in HB concentration compared to negative control group.

2.2. Liver function tests

Squabs of positive control group exhibited significant increase in serum (ALT, AST, GGT and Bilirubin) and a non-significant increase in ALP. But squabs of Chlorine treated group, UV treated group and Ozone treated group exhibited anon significant increase in serum ALT, AST, Bilirubin, GGT and ALP compared to negative control group.

2.3. Lipid profile tests

Squabs of positive control group exhibited significant increase in serum Cholesterol and Triglycerides compared to negative control group. But squabs of Chlorine treated group, UV treated group and Ozone treated group exhibited anon significant increase in Cholesterol and TG compared to negative control group.

2.4. Brain function tests

Squabs of positive control group exhibited significant increase in serum (MAO, ChE and 5́–NT) and a non-significant increase in Creatine kinase compared to negative control group. But squabs of Chlorine treated group, UV treated group and Ozone treated group exhibited anon significant increase in MAO, ChE., CK and 5́ –NT. compared to negative control group.

2.5. Serum immunoglobulin tests

Squabs of positive control group exhibited significant increase in serum IgM and a non-significant increase in IgG and IgA compared to negative control group. But squabs of Chlorine treated group, UV treated group and Ozone treated group exhibited anon-significant increase in serum IgM, IgA and IgG compared to negative control group.

2.6. Serum proinflammatory cytokine tests:

Squabs of positive control group exhibited significant increase in serum IL-2 and IL-6 compared to negative control group. But squabs of Chlorine treated group, UV treated group and Ozone treated group exhibited anon-significant increase in serum IL-2 and IL-6 compared to negative control group.

4. DISCUSSION

A variety of bacterial, protozoan and viral pathogens is transmitted via water, and infection may occur through inhalation, ingestion, or topical contact with reused water. A key route of transmission is thought to be through aerosol generation during the agitation of water, which enables the movement of pathogenic microorganisms, (Fannin et al., 1985; Goldmann, 2000) facilitating their transmission by inhalation, and their transport onto surfaces, which may subsequently come into contact with, or be ingested by, an individual, (Beggs, 2003).

Table: Hematological and biochemical changes in all group of pigeons.

Groups / Tests	Negative control (1)	Positive control (2)	Chlorine treated (3)	Ozone treated (4)	UV. treated (5)
HB. Conc. (g/dl)	11.35^{A} ±0.16	8.49^{B} ±0.29	10.26^{C} ±0.19	10.56^{CD} ±0.13	10.89^{DA} ±0.12
ALT activity (IU/l)	14.81^{A} ±.462	33.84^{B} ±.384	16.78^{C} ±.663	15.70^{AC} ±.665	14.34^{A} ±.310
AST activity (IU/l)	25.67^{A} ±1.17	51.11^{B} ±1.82	27.33^{A} ±1.12	26.08^{A} ±1.18	25.60^{A} ±1.15
GGT activity (IU/l)	18.57^{A} ±.48	24.49^{B} ±.77	20.29^{C} ±.41	19.13^{AC} ±.42	18.28^{A} ±.44
Bilirubin conc. (mg/dL)	1.191^{A} ±.003	1.52^{B} ±.003	1.34^{C} ±.003	1.28^{D} ±.004	1.198^{A} ±.003
Alkaline phosphatase activity (U/l)	264.99 ±2.14	265.54 ±2.14	265.09 ±2.15	265.05 ±2.15	264.97 ±2.14
Cholesterol conc. (mg/dl)	162.497^{A} ±3.5590	196.832^{B} ±2.5396	171.894^{C} ±2.4680	166.67^{AC} ±2.306	163.06^{AC} ±4.1776
Triglycerides conc. (mg/dl)	86.50^{A} ±.341	125.50^{B} ±.341	110.20^{C} ±.940	101.60^{D} ±.890	97.40^{D} ±.371
IgA (ng/ml)	191.70 ±1.606	193.20 ±.566	192.80 ±.614	192.70 ±1.649	191.80 ±.517
IgG (ng/ml)	214.20 ±.388	215.10 ±.339	214.80 ±3.382	214.60 ±.339	214.60 ±.339
IgM (ng/ml)	126.90^{A} ±.276	222.90^{B} ±.276	156.90^{C} ±.276	135.90^{D} ±.276	129.90^{E} ±.276
IL-2 (pg/ml)	0.389^{A} ±.009	4.149^{B} ±.009	1.575^{C} ±.074	1.316^{D} ±.029	1.189^{E} ±.027
IL-6 (pg/ml)	6.024^{A} ±.004	12.819^{B} ±.042	6.503^{C} ±.030	6.236^{D} ±.0067	6.129^{E} ±.004
Monoamine oxidase	19.21^{A} ±.001	24.80^{B} ±.521	20.82^{C} ±.001	20.34^{CD} ±.002	19.70^{AD} ±.002
Cholinesterase activity	7.68^{A} ±.004	10.95^{B} ±.470	8.86^{C} ±.004	8.59^{CD} ±.058	8.195^{AD} ±.004
5́-NT activity	3.22^{A} ±.003	4.98^{B} ±.003	4.12^{C} ±.003	3.53^{D} ±.003	3.33^{E} ±.003
Creatine Kinase	680.90^{A} ±.276	683.90^{B} ±.276	682.90^{C} ±.276	681.90^{D} ±.279	680.90^{A} ±.276

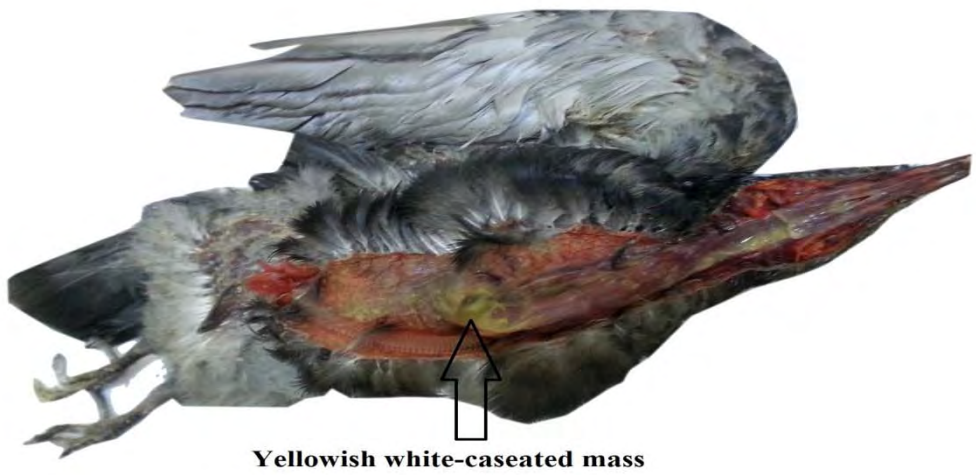

Figure: A yellowish white-caseated mass in the buccal cavity of an infected pigeon with *T. gallinae*

Trichomonad is a protozoan that able to survive in water and moist grain, and it has been suggested that contaminated water and seed sources may be significant in the transmission of trichomonads. Contamination of water and seed sources may occur when an infected bird drops water and seed, (Stabler 1954, Kocan 1969, Honigberg 1979). Disinfection or inactivation process is a process which rendering a pathogen harmless (not necessarily killing it), (USEPA, 2004). Data revealed that, Squabs of positive control group exhibited significant decrease in HB. Concentration compared to negative control group. However, squabs of Chlorine, UV and Ozone treated groups exhibited anon-significant decrease in HB conc. compared to negative control group. These observations were similar to the reported studies of (El-Sayed, 2005) who recorded that, infected squabs of Trichomonas gallinae suffered from macrocytic hypochromic anemia. In addition, (Sekhar and Sinha, 1986) observed anemia, lymphocytosis, leukocytosis and eosinophilia in trichomonosis. Data revealed that, Squabs of positive control group exhibited significant increase (ALT, AST, GGT, MAO, ChE, 5`-NT and Bilirubin) and a non-significant increase in ALP and CK. However, squabs of Chlorine, UV and Ozone treated groups exhibited a non-significant increase in serum ALT, AST, Bilirubin, GGT and ALP compared to negative control group. These observations were came in the same line with the reported studies of (Lumeij, 1987) who reported that, Trichomonas gallinae induced hepatic necrosis in Columbiformes and Falconiformes causes elevated plasma Glutamate dehydrogenase (GLDH), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Gamma glutamyl transferase (GGT). Similarly, these results come in the same line with (Seddiek et al., 2014) who recorded that, infected squabs with Trichomonas gallinae there was increased levels of ALT and AST. Squabs of positive control group exhibited significant increase in serum Cholesterol and TG compared to negative control group. However, squabs of Chlorine treated group, UV treated group and Ozone treated group exhibited a non-significant increase in Cholesterol and TG compared to negative control group. The recorded results agreed with those reported by (Seddiek et al. 2014) who recorded that, in vivo study the infected squabs with Trichomonas gallinae there was increased levels of ALT, AST and cholesterol. Squabs of positive control group exhibited significant increase in serum (IL-2, IL-6 and IgM) compared to negative control group. Nevertheless, squabs of Chlorine, UV and Ozone treated

groups exhibited anon-significant increase in serum IL-2, IL-6 and IgM. The recorded results agreed with those reported by (Dispenzieri et al., 2001) who reported that, Serum immunoglobulin levels are determined routinely in clinical practice because they provide key information on the humoral immune status. High immunoglobulin levels are observed in liver diseases, chronic inflammatory diseases, haematological disorders, infections and malignancies.

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التأثيرات الكيميائية الحيوية للتريكوموناس جاللينى في الحمام المربي على مياه ترع الري والمياه المعالجة بمضادات الأوليات.

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الملخص العربي

تم ايجاد الطفيل في فم إحدى الحمامات المصابة به وتم عمل مزرعة له على ميديا خاصة تسمي جلوكوز سيرم ميديا. ثم تم صب الميديا على مجاميع المياه كالتالي: * مياه تحتوي على طفيل التريكوموناس جاللينى لم يتم معالجتها. مياه تحتوي على طفيل التريكوموناس جاللينى تم معالجتها بالكلور 1 مجم/لتر. مياه تحتوي علي طفيل التريكوموناس جاللينى تم معالجتها بالأشعة فوق البنفسجية. مياه تحتوي علي طفيل التريكوموناس جاللينى تم معالجتها بغاز الأوزون 0.1 مجم/لتر. وقد أجريت هذه الدراسة لأمد قصير أربعة عشر يوما على عدد خمسون من زغاليل الحمام تتراوح أعمارهم من أسبوعين الي ثلاث أسابيع تقريبا وقد تم تقسيمهم كالآتي: المجموعة الأولـــي: 10 زغاليل تم تربيتها علي مياه مقطرة خاليه من الطفيليات كمجموعة ضابطة. المجموعة الثانيـــة: 10 زغاليل تم تربتها على مياه تحتوي علي الطفيل غير معالجه. المجموعة الثالثـــة: 10 زغاليل تم تربتها على مياه معالجة عن طريق الكلور. المجموعة الرابعـــة: 10 زغاليل تم تربتها على مياه معالجة عن طريق الاشعة فوق البنفسجية. المجموعة الخامسة: 10 زغاليل تم تربتها على مياه معالجة عن طريق غاز الأوزون. هذا وقد تم تجميع العينات في نهاية الاسبوع الثاني وذلك لإجراء الفحوصات التالية: قياس نسبة الهيموجلوبين بالدم. انزيمات الكبد *(ALT, AST, GGT,ALP)* ونسبة الصفراء في الدم. مستوي الدهون في المصل الكوليسترول الكلي والدهون الثلاثية. انزيمات المخ *(MAO,5`-NT,ChE,CK)* في المصل. مستوي الأجسام المضادة في المصل (*IgA, IgG, IgM*). مستوي السيتوكينات *IL-2,IL-6* في المصل. وجد اختلال معنوي في وظائف الكبد والمخ وايض الدهون وانخفاض عالي المعنوية في نسبة الهيموجلوبين بالدم بالإضافة الي حالة التهاب حادة تتضح من الارتفاع عالي المعنوية في مستوي السيتوكينـــات *IL-2 and IL-6* وفي مستوي الأجسام المضادة في المصل (*IgM*) في الطيور المصابة بطفيل التريكوموناس جاللينى بينما لم يحدث اختلال في وظائف الكبد والمخ وايض الدهون ولم يحدث نقص ملحوظ في نسبة الهيموجلوبين بالدم ولم يحدث ارتفاع ملحوظ في مستوي السيتوكينـــات وفي مستوي الأجسام المضادة للطيور المربي علي المياه المعالجة بمضادات الاوليات (الكلور- الاوزون- الاشعة فوق البنفسجية) مقارنة بالمجموعة الضابطة.

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