In the Name of God

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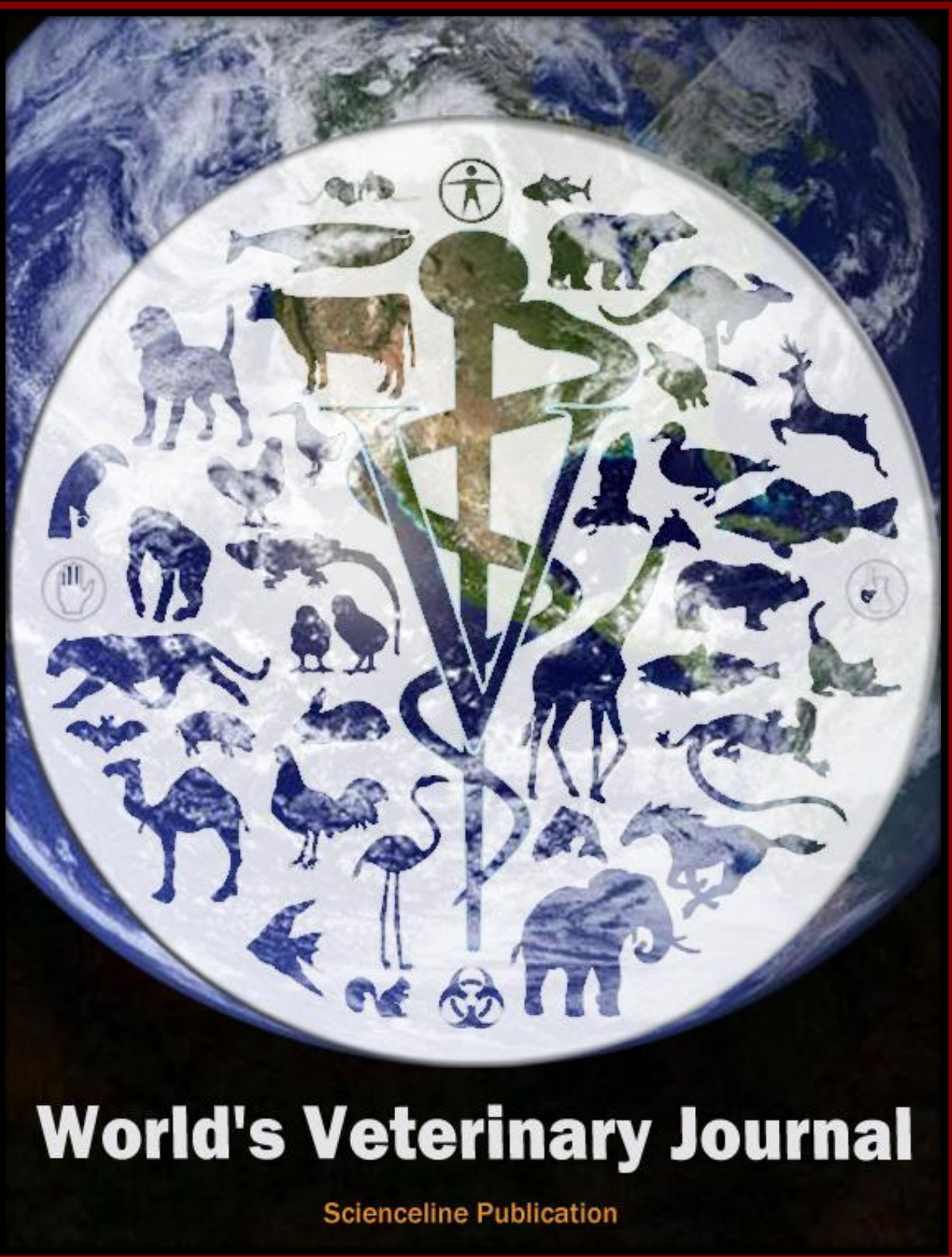

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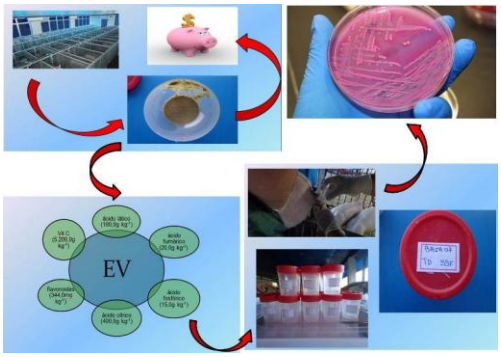
Volume 8 (1); March 25, 2018

Research Paper



Performance and Microbiological Profiles of Piglets Fed with Diets Enriched with Bio-flavonoids and Ascorbic Acid.

de Oliveira MF, Rigon Rossi CA, Shardong Lucca M, Soares M, de Oliveira V, Dornelles J, de Lima Schlösser LM and Guilherme Gräf C.

World Vet. J. 8(1): 01-08, 2018; pii:S232245681800001-8

ABSTRACT
The objective of this study was to evaluate the performance and microbiological profile of 40 piglets (females and males) in the nursery phase. The experimental design was completely randomized, with four treatments, five replicates and sex as a blocking factor. The treatments were distributed in: T1 (control); T2 (Plant Extract as PE, 500 ppm); T3 (Amoxicillin as A, 20 mg kg^{-1}) and T4 (PE+A, 500 ppm + 20 mg kg^{-1}). There was no influence ($P > 0.01$), between treatments for both the initial and the final weight and average daily gain, but the control group males had an average daily feed intake of 1.8% or higher ($P < 0.01$) compared to other treatments. The total count control bacterial colonies were 35.9%, 70.9 % and 63.8 % higher ($P < 0.01$) to treatment with A, PE+A and PE, respectively. For MacConkey test, the treated group A was 88.44 %, 91.78 % and 56.50 % higher ($P < 0.01$) compared to PE+A, PE and control, respectively. The antibiogram of 48 stool samples had shown that Amoxicillin disk were at 85.7 %, 72.7 %, 44.5 % and 100 % resistant in the control treatments, PE, A and PE+A respectively. The bioflavonoids and ascorbic acid and the interaction with amoxicillin did not alter the performance of pigs in the nursery phase but had reduced the presence of bacterial colonies.
Key words: Amoxicillin, Bacterial colonies, *E. coli,* Nursery, Plant extract

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Research Paper

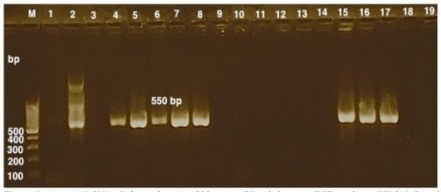

Figure 3. Agarose (1.5%) gel electrophoresis of *hla* gene of *Staphylococcus* PCR products (550 bp). Lane M: 100 bp DNA marker, Lanes: 1, 3, 9-14 and 18-19: negative isolates, lanes: 2, 4-8 and 15-17: positive isolates.

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To cite this paper: Ali MM, Helmy SM, El Desouky IE and Asfour HA (2018). Molecular Characterization of *Staphylococci* Isolated from Cattle with Mastitis. *World Vet. J.* 8(1): 09-18; http://wvj.science-line.com

Molecular Characterization of *Staphylococci* Isolated from Cattle with Mastitis.

Ali MM, Helmy SM, El Desouky IE and Asfour HA.

World Vet. J. 8(1): 09-18, 2018; pii:S232245681800002-8

ABSTRACT
This study was carried out in order to investigate the occurrence of some virulence genes of *Staphylococci* isolated from cattle with mastitis. A total number of 133 milk samples (45 from clinical mastitis and 88 from subclinical mastitis) were collected from dairy cattle in Kafr El-Sheikh and EL Gharbia Governorates, Egypt. The samples were examined for the presence of *Staphylococci* by classical bacteriological methods and were further characterized geno-typically. A total of 41 *Staphylococcus* isolates were recovered from cattle with mastitis with an incidence of 30.8%. Among the isolates, 21(15.8%) of *S. aureus* [6 from clinical mastitis (13.3%) and 15 from subclinical mastitis (17%)] and 20 (15%) isolates of CNS [8 from clinical mastitis (17.7%) and 12 from subclinical mastitis (13.6%)] were identified phenotypically. All isolates were screened for the detection of binding protein A (*spa*-X*),* haemolysine type A (*hla),* Haemolysine type B (*hlb),* and toxic shock syndrome (*tsst*-1*)* by PCR. The obtained results revealed that the *spa* Xgene was detected in all *Staphylococcus* isolates recovered from subclinical mastitis while in clinical mastitis was detected with an incidence of 42.9%. Haemolysine type A was detected in clinical and subclinical mastitis with an incidence of 71.4% and 70% respectively, while haemolysine type B was detected in clinical and subclinical mastitis with an incidence of 28.5% and 40% respectively. Toxic shock syndrome was not detected in any of the isolates. The data in the study provided an overview on the distribution of some virulence genes related to *Staphylococci* isolated from cattle with mastitis in Egypt.
Key words: Cattle, Mastitis, *Staphylococci*, Virulence gene, PCR

[Full text-PDF] [MOBI] [ePUB] [AZW3]

Research Paper

Parasitic Infection with Emphasis on *Tylodelphys* spp. as New Host and Locality Records in Nile Perch; *Lates niloticus* from Lake Nasser, Egypt.

Hamouda AH, Sorour ShS, El-Habashi NM and El-Hussein AA.

World Vet. J. 8(1): 19-33, 2018; pii:S232245681800003-8

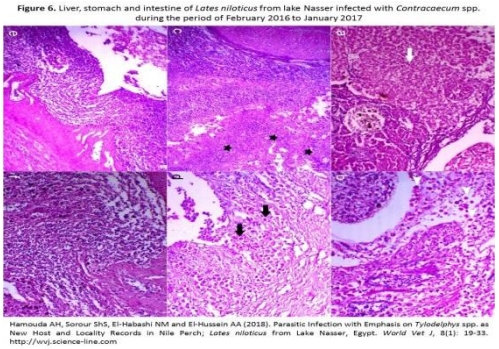

Figure 6. Liver, stomach and intestine of *Lates niloticus* from lake Nasser infected with *Contracaecum* spp. during the period of February 2016 to January 2017

Hamouda AH, Sorour ShS, El-Habashi NM and El-Hussein AA (2018). Parasitic Infection with Emphasis on *Tylodelphys* spp. as New Host and Locality Records in Nile Perch; *Lates niloticus* from Lake Nasser, Egypt. *World Vet J*, 8(1): 19-33. http://wvj.science-line.com

ABSTRACT

A total number of 200 *Lates niloticus* were collected alive from several and various localities at Lake Nasser in Aswan governorate, to investigate the prevailing parasites that infect this fish species. All the examined fish were positive for one or more parasites, three trematodes of two families were identified: *Diplectanum simile*, *Diplectanum lacustris* and *Tylodelphys* spp. (recorded for the first time in *Lates niloticus* representing new host and locality records), two nematodes of two families: *Philometra ovata* and L_3 larvae of *Contracaecum* spp.(has zoonotic importance), one acanthocephalan parasite: *Rhadinorhynchus niloticus*, two crustaceans parasites of one family: *Ergasilus kandti* and *Ergasilus latus*, while no cestodal infections were recorded at all. The prevalence of trematodes was at 95% meanwhile the nematodes were at 100% in addition to the acanthocephalan parasite was at 24.5% as well, crustaceans parasites were at 69.5%. This study evaluated clinical signs, postmortem examinations, parasitological examinations, seasonal prevalence and histopathological investigations of infected fish in addition to the relation between fish age and parasitism was also described. This study builds on our current understanding of different parasites infecting the wild *Lates niloticus* and provides novel information on the patterns of the isolated parasites and also serves to reassure the consumers that the musculature (the edible part) of the fish was free from any parasitic infections and safe for human consumption provided that the fish must be eviscerated as soon as possible after being caught and adequately cooked.

Key words: *Lates niloticus*, Nile perch**,** *Tylodelphys* spp., *Philometra ovata*, Pathology, Lake Nasser

[Full text-PDF] [MOBI] [ePUB] [AZW3]

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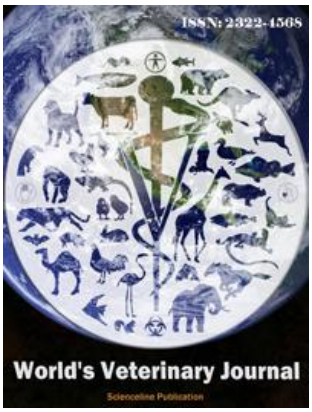

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Performance and Microbiological Profiles of Piglets Fed with Diets Enriched with Bio-flavonoids and Ascorbic Acid

Magali Fernandes de Oliveira[1], Carlos Augusto Rigon Rossi[1]*, Matheus Shardong Lucca[1], Marcelo Soares[1], Vladimir de Oliveira[2], Julianni Dornelles[1], Luara Medianeira de Lima Schlösser[1] and **Cristian Guilherme Gräf[2]**

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ABSTRACT

The objective of this study was to evaluate the performance and microbiological profile of 40 piglets (females and males) in the nursery phase. The experimental design was completely randomized, with four treatments, five replicates and sex as a blocking factor. The treatments were distributed in: T1 (control); T2 (Plant Extract as PE, 500 ppm); T3 (Amoxicillin as A, 20 mg kg^{-1}) and T4 (PE+A, 500 ppm + 20 mg kg^{-1}). There was no influence (P>0.01), between treatments for both the initial and the final weight and average daily gain, but the control group males had an average daily feed intake of 1.8% or higher (P<0.01) compared to other treatments. The total count control bacterial colonies were 35.9%, 70.9 % and 63.8 % higher (P<0.01) to treatment with A, PE+A and PE, respectively. For MacConkey test, the treated group A was 88.44 %, 91.78 % and 56.50 % higher (P<0.01) compared to PE+A, PE and control, respectively. The antibiogram of 48 stool samples had shown that Amoxicillin disk were at 85.7 %, 72.7 %, 44.5 % and 100 % resistant in the control treatments, PE, A and PE+A respectively. The bioflavonoids and ascorbic acid and the interaction with amoxicillin did not alter the performance of pigs in the nursery phase but had reduced the presence of bacterial colonies.

Key words: Amoxicillin, Bacterial colonies, *E. coli*, Nursery, Plant extract

ORGINAL ARTICLE
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INTRODUCTION

The continuous overcoming of the technical difficulties is a challenge that marks the lives of modern pig farming. There were numerous contributions ranging from genetic improvement, the improvement of knowledge about nutrition and health, ambience, facilities and reproduction. Nevertheless, there are several challenges to be overcome in all sectors of pork production, because even with the modern technologies available, the piglets are still suffering from the enteropathies (Anami et al., 2008).

Diarrhea, and other diseases that affect the digestive tract of pigs have many factors and the major contributors involved are the *Clostridium perfringens* type A or type C, *Escherichia coli* enterotoxigenic, *Isospora suis*, *rotavirus* and transmissible gastroenteritis virus of pigs (Yaeger et al., 2007; Hur and Lee, 2012). In a brief consideration of the occurrence of diarrhea in pigs, which determines the importance of these episodes are factors such as the number of patients, the course of the disease, the degree of dehydration of the affected piglets, the specific mortality due to a problem, the repetition of episodes in different lots and the quantities and efficiency of drugs and vaccinations in course (Barcellos et al., 2011). Diarrhea occurs with clinical signs such as loss of solutes and water, electrolyte depletion, acid-base imbalance and dehydration, which can be fatal if not treated properly (Zlotowski et al., 2008). The treatments are difficult, facing high and often inefficient costs, but prevention and proper management are effective ways to reduce the incidences of diarrhea. The need to ensure the zootechnical and economic results of pig production encouraged the

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routine incorporation of antibacterial (growth promoters) in feed intended for stages of the production process. Accordingly, new alternatives to ensure animal performance, quality of final product and reduce undesired waste to consumer health are being scientifically considered.

Research has been conducted with prebiotics, enzymes, organic acids and plant extracts (PE). Organic acids (OAs) have been widely used in pig diets, acting as a promising substitute for antibiotics and antibiotic growth promoters (AGP) (Lei et al., 2017; Long et al., 2018). Previous studies showed that both free and encapsulated organic acids supplementation improved the performance of weaned piglets and also intestinal morphology and health (Diao et al., 2016). The PE have been represented by phenolic compounds (flavonoids or bioflavonoids), and ascorbic acid. The bioflavonoids are natural antioxidants with anti-inflammatory action, anti-microbial, antiallergics and immune-stimulating (Cushnie & Lamb, 2005). Ascorbic acid takes part in several metabolic processes, such as the formation of collagen, synthesis of epinephrine, corticosteroids and bile steroids (Pion et al., 2004). Besides enzyme cofactor, ascorbic acid participates in the redox processes, enhancing iron absorption and inactivation of free radicals (Padayatty et al., 2003). The benefits of therapeutic use of ascorbic acid in pigs are observed in the performance, pre-slaughter stress and meat quality (Pion et al., 2004).

The individual properties and synergistic action of its active ingredients, the use of PE can enhance the immune response of piglets in nursery phase. Although there is positive information related to the synergy of the constituents of PE, its use in the control of clinical signs of diarrhea in piglets are weak and inconclusive. The objective of this work was to evaluate the performance and the microbiological profile of piglets in the nursery phase fed with diets containing bio-flavonoids and ascorbic acid.

MATERIALS AND METHODS

The animals were housed in Sector Swine Department of Animal Science UFSM, Brazil. The experiment was performed in the period 2 to 23 December 2016. The experimental units had an average weight of ± 5.89 kg. The nursery shed had 48 raised bays at 0.40m from the ground, with leaked plastic floor with $2m^2$ of area per bay 48 raised bays. The stalls were equipped with semi- automatic feeders and drinker's pacifier type, with height adjustment. The shed had automatic drive curtains, a set of four exhaust fans and air conditioning.

The animals upon arrival were weighed, identified and distributed in the treatments. Forty animals housed were used in 40 stalls, 20 females and 20 males weaned, arranged in a completely randomized design with four treatments, five replicates and used sex as blocking factor.

Treatments

The treatments were as follows, respectively: T1 (control); T2 (PE, 500 ppm); T3 (Amoxicillin, 20 mg kg^{-1}) and T4 (PE+A, 500 ppm + 20 mg kg^{-1}). The PE consists of lactic acid (180 g kg^{-1}), vitamin C (5.200 g kg^{-1}), flavonoids (344 mg kg^{-1}), citric acid (400 g kg^{-1}), phosphoric acid (15 g kg^{-1}), fumaric acid (20 g kg^{-1}). The Amoxicillin (50 g) was used at a dose of 20 mg per kilogram of weight. The animals were subjected to constant clinical evaluations regarding the degree of hydration and diarrhea symptoms. The minimum and maximum temperature was recorded twice daily. The animals had consumed a commercial iso-nutritive diet, following the nutritional requirements of NRC (2012) (Table 1), the animals were fed at will and had ad libitum access to water. The weight gain data were obtained weekly and weights of individual animals. The daily feed intake was obtained by according to the weighing the ration provided, that were remaining as daily leftovers present in the feeders. Feed conversion was estimated from the previous variables.

Ethical approval

This work was approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Santa Maria (UFSM), Santa Maria, RS, Brazil.

Microbiological profile

To evaluate the microbiological profile, animal feces were collected, identified and sent to the Bacteriology Laboratory (LABAC) of the Federal University of Santa Maria (UFSM) for counting bacterial colonies. The fecal samples were taken on days the 7th, 14th and 21st of the experiment. PCA was used to count colonies (Plate Count Agar) and MacConkey. Further biochemical tests were performed (oxidation and fermentation of sugars (GOF), Indol production (SIM), use of sugar (TSI) and urease) for the identification of *E. coli*. The colonies were stored in glycerol for subsequent culture and sensitivity of 48 preserved specimens. The samples passed through subculture in VBR and then taken to an oven for 24 hours at 37ºC. After this period, with platinum loop, some colonies were added to Muller Hilton broth (MH) and thereafter on MH agar and added to the antibiotic disks to be routed to incubator for 24 hours at 37°C. After 24 hours the reading made with a transparent millimeter ruler to determine the diameter in mm for each inhibition

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zone. The following discs were used for susceptibility testing: Polymyxin (30 ug), Cefepine (30 µg), Amoxicillin (10 mg), Neomycin (30 µg), Norfloxacin (10 mg), Ceftriaxone (30 µg), Meropenem (10 mg) and Ampicillin (10 mg).

Table 1. Proximate analysis of diets supplemented piglets in nursery phase diets containing bioflavonoids and ascorbic acid

Ingredients	Pre-initial I*	Initial I*
Crude Protein (g kg^{-1})	180	177.99
Humidity (g kg^{-1})	120	120
Formic Acid (mg kg^{-1})	325	-
Folic Acid (mg kg^{-1})	-	0.60
Pantatenic Acid (mg kg^{-1})	-	13.65
Biotin (mg kg^{-1})	-	75
Cobalt (mg kg^{-1})	-	19.95
Lactic Acid (mg kg^{-1})	760	-
Calcio Pantothenate (mg kg^{-1})	11	-
Fruit Aroma (mg kg^{-1})	30	-
BHT (mg kg^{-1})	97	-
Calcio (mg kg^{-1})	8,000	8
Calcio (mg kg^{-1})	5,000	6.25
Cuprum (mg kg^{-1})	8	0.6
Colina (mg kg^{-1})	-	349
Etoxiquin (mg kg^{-1})	-	25
Ethereal extract (mg kg^{-1})	25	27.6
Iron (mg kg^{-1})	79	112
Gross fiber (g kg^{-1})	35	22.6
Phosphorus mg kg^{-1})	3,500	5.4
Halquinol (mg kg^{-1})	120	-
Iodine (mg kg^{-1})	0.49	1.95
Lisina (g kg^{-1})	12	6,175
Manganes (mg kg^{-1})	30	62.25
Mineral Material (g kg^{-1})	60	33.38
Metionina (mg kg^{-1})	5,000	3,394
Niacina (mg kg^{-1})	18	33
Selenio (mg kg^{-1})	0.25	0.3
Sodio (mg kg^{-1})	1,800	0.24
Treonina (mg kg^{-1})	1,800	-
Triptofano (mg kg^{-1})	790	-
Vitamin A (UI kg^{-1})	6,400	15,900
Vitamin B12 (mcg kg^{-1})	30	30
Vitamin B1 (mg kg^{-1})	-	1.2
Vitamin B2 (mg kg^{-1})	4	8.4
Vitamin B6 (mg kg^{-1})	2	3.39
Vitamin D3 (UI kg^{-1})	1,200	32,400
Vitamin E (UI kg^{-1})	30	39
Vitamin K3 (mg kg^{-1})	1	1.65
Zinco (mg kg^{-1})	2,500	2,250

Basic product composition: folic acid, nicotinic acid, calcium carbonate, sodium chloride, choline chloride, etoxiquin, transgenic soybean meal, ground corn, phytase, dicalcium phosphate, halquinol, potassium iodate, zinc oxide, calcium pantothenate, sodium selenite, cobalt sulfate, copper sulfate, iron sulfate, manganese sulfate, vitamin A, vitamin B1, B2, B6, B12, D3, E and K.

Data Analysis

The data were submitted to variance analysis by the GLM procedure in the 5% level of significance. The effects included in the analytical model were treatments (T) and week (S). Any differences between the means were compared by Tukey test ($P<0.05$). Statistical analyzes were performed using the statistical software Minitab version 15 (Mckenzie and Goldman, 2010). The antibiotic susceptibility data were assessed through sensitivity percentage, intermediate susceptibility and resistance to antibiotics.

RESULTS

Animal performance did not differ ($P>0.01$) between treatments for both the initial and the final weight and average daily gain. However, the control group males had an average daily feed intake of (CDMR) 1.8% higher ($P<0.01$) to other treatments and feed conversion (FC) 2.11% higher ($P<0.01$) compared to treatment with PE (Table 2). The total count of bacterial colonies in the control group were at 35.9%, 70.9% and 63.8% higher ($P<0.01$) to treatment with A, PE+A and PE, respectively. The count of small colonies in the control group was 72.3%, 75.99% and 75.35% higher ($P<0.01$) to treatment with A, PE+A and PE, respectively. The average colony count of treatment with A was 58.27%, 75.37% and

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99.82% higher (P<0.01) to treatment with PE, PE+A and the control group, respectively. Regarding the large colonies there were no differences (P>0.01) between treatments. Therefore, in the count MacConkey way, the treated group A was 88.44%, 91.78% and 56.50% higher (P<0.01) to treatment with PE+A, PE and control, respectively (Table 3).

In assessing the susceptibility testing (percentage data), it was observed that antibiotics (Cefepine, CPM; Ceftriaxone, CRO and Merepenem, MPM) showed 100% sensitivity independent of the treatments. The polymyxin showed 100% resistance in the control treatments, PE, and PE+A, but 66.6% of resistance in the treatment A. The antibiotic susceptibility to the amoxicillin disc had showed 85.7%, 72.7%, 44.5% and 100% resistance in the control treatments, PEA and PE+A, respectively (Table 4). The disks with ampicillin and Norfloxacin showed 88.8% and 88.8% strength, respectively in treatment A. In summary, we can see that the antibiogram with Amoxicillin discs, Norfloxacin and Ampicillin showed higher percentage of resistance in dependent of treatments. Still, we can see that the antibiogram with neomycin discs showed 100% of intermediate sensibility in treatment with PE+A and0% resistance in the other treatments.

Table 2. Performance of piglets in nursery phase fed diets containing bioflavonoids and ascorbic acid

Treatments (T)	Weight In		Weight Fi		ADFI		DWG		CF	
	M	F	M	F	M	F	M	F	M	F
Control	5.69	5.55	9.01	8.90	0.53[a]	0.53	0.36	0.36	1.42[a]	1.41
Plant Extract (PE)	5.78	6.61	9.16	9.98	0.52[b]	0.52	0.36	0.36	1.39[b]	1.39
Amoxicillin (A)	5.88	6.61	9.22	9.92	0.52[b]	0.52	0.36	0.36	1.39[ab]	1.41
PE+A	5.41	5.67	8.76	8.79	0.52[b]	0.52	0.36	0.36	1.40[ab]	1.41
RSD	0.56	0.54	0.89	0.83	0.01	0.01	0.01	0.01	0.01	0.03
Probability										
T	0.87	0.91	0.23	0.17	0.01	0.08	0.85	0.94	0.01	0.54
R	0.01[1]	0.01[2]	0.69	0.70	0.01[3]	0.07	0.11	0.54	0.01[4]	0.21

Weight In= initial weight; Weight Fi= final weight; ADFI= average daily feed intake; DWG= daily weight gain; CF= conversion food; RSD = residual standard deviation; R= repeat; M= male; F= female; Regression equations for [1]Weight In males: (Weight In=4.65+0.59R) =; [2]Weight In females: (Weight In=6.03+0.10R); [3]ADFI males: (ADFI=0.52+0.0008R); [4]CF machos: (CF=1.38+0.012R).

Table 3. Count of bacterial colonies of fecal content in initial phase of piglets nursery fed diets enriched with bioflavonoids and ascorbic acid

Treatments	Count (UFC mL^{-1})				
	Total Count	Small Colony	Medium Colony	Big Colony	mC
Control	684797437.5[a]	507353125[a]	515000[c]	24855375	24855375[b]
Plant Extract (PE)	247453000[c]	125062500[b]	121118125[b]	1334875	4693963[b]
Amoxicillin (A)	438725000[b]	140493750[b]	290243750[a]	250000	57148625[a]
PE+A	198959562.5[c]	121786875[b]	71472688[c]	2668750	6604125[b]
RSD	179	129	142	288	239
Probability					
T	0.01	0.01	0.01	0.14	0.01
W	0.01[1]	0.01[2]	0.01[3]	0.01[4]	0.01[5]

Atb= antibiotic; RSD=residual standard deviation; W=Week; mC= MacConkey Agar; Regression equation for week evaluation: [1]W=2.80 - 0.000000 The total colony count; [2]W=2.84 - 0.023000 Count small colonies;[3]W=2.44 + 0.031000 Count medium colonies; [4]W=2.72 - 0.0420000 Count large colonies; [5]W=2.62 - 0.063000 mC.

Table 4. Antibiogram (sensitivity in %) of piglets from stool samples in nursery phase fed diets containing bioflavonoids and ascorbic acid

Treatments	Sensitivity	Polym	CPM	AMO	NEO	NOR	CRO	MPM	AMP
Control	S	100	100	14.3	85.7	100	100	100	14.3
	IS	0	0	0	14.3	0	0	0	0
	R	0	0	85.7	0	0	0	0	85.7
Plant Extract (PE)	S	100	100	27.3	54.5	36.4	100	100	27.3
	IS	0	0	0	45.5	9.1	0	0	0
	R	0	0	72.7	0	54.5	0	0	72.7
Amoxicillin (A)	S	66.6	100	33.3	55.5	11.2	100	100	11.2
	IS	22.2	0	22.2	44.5	0	0	0	0
	R	11.2	0	44.5	0	88.8	0	0	88.8
PE+A	S	100	100	0	0	0	100	100	33.3
	IS	0	0	0	100	0	0	0	0
	R	0	0	100	0	100	0	0	66.6

Polym= polymyxin; CPM= Cefepine; AMO= Amoxicillin; NEO= Neomycin; NOR= Norfloxacin; CRO= Ceftriaxone; MPM= Merepenem; AMP= Ampicillin; S= Sensitive; IS= Intermediate sensitivity; R= Resistant

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DISCUSSION

Currently, the nutritional aspects involving the plant extracts have been measured by the antimicrobial activity they have on the enzyme systems, cell structures and biological molecules. However, it is interesting to note that the biological effects of natural antioxidants are enhanced by interactions between the constituents of the formula (Middleton et al., 2000). For example, bioavailability and efficiency of vitamin C and bioflavonoids are lower than if they were administered alone (Navarro et al., 2008). Among the various actions, antioxidants protect the immune system. The bioflavonoids modulate inflammatory responses, such as inhibition of PGE2 inhibition of IgE and membrane myelin phagocytosis in multiple sclerosis process (Flórez, 2002). Currently, the acidifying is used in the diets of pigs in the early stages of growth, aiding in performance after weaning (Miguel, 2008).

In our study, we observed a higher average daily feed intake of males in the control group and consequently lower feed efficiency, compared to the other treatments. In another study, one of the constituents of the formula for PE, more specifically fumaric acid was used in the diet of piglets in post- weaning, it had improved animal performance, both the weight gains and feed conversion as well as increased feed intake (Teixeira et al., 2003). Already using 1.5 to 3.0 % citric acid in piglet's diet post weaning, showed no improvement in weight gain and feed efficiency (Radecki et al., 1988). Already, Xu et al. (2018) testing the combination of OAs and oils essentials didn't show a larger positive effect on intestinal health than when supplied individually, but it may increase the growth performance according to the complementary effects of OA and oils essentials in weaned piglets. Therefore, the responses of the performance characteristics and the apparent digestibility coefficients of nutrients, acidifying front of supplementation are variable and contradictory (Miguel, 2008).

Several hypotheses are suggested regarding the mechanism of action of acidifying and between the reduction in stomach pH, changes in intestinal microflora (by control bactericide) or bacteriostatic, improved digestibility and nutrient retention (Miguel, 2008). It is important to remember that the secretion of hydrochloric acid in young piglets is limited due to insufficient production of hydrochloric acid. This was observed in another study, which assessed liquid diets fermented or not, and stressed that reducing the pH favor the use of short-chain fatty acids (organic acids) and control of enterobacteria (Canibe et al., 2007). Accordingly, the use of acidifiers in the diets of weaning piglets in the post may serve as an adjuvant to control pH of the stomach and assist in the digestion of food grain-based and vegetable bran (Gallo et al., 2003). However, we note that the most consistent results are relative to antimicrobial power of acidifying.

This power, in most cases occurs when stomach pH has decreased. One of microbial control mechanisms refers to the capacity of that acidifying have to change the pH of the environment due to its potential dissociation (pKa) between the dissociated and non- dissociated (Partanen and Mroz, 1999). The absorption of organic acids takes place faster when the luminal pH value is smaller than pKa of the acids. The pKa of an acid is the pH at which 50% of the acid is in the ionized form, being determined by the negative logarithm of the acid ionization constant, or Ka, which in turn indicates the acid strength, so its tendency to donate protons. For be expressed logarithmically, one pH unit above the pKa of an acid indicates that 90% of the acid is in the non-dissociated form and with two pH units above the pKa, 99% of the acid will not be dissociated and pKa of the most acidic are between 3 and 5 (Bellaver and Scheuermann, 2004; Thompson and Hinton, 1997).

When the acid is in the ionized form can diffuse freely through the semipermeable membrane of the microorganism to its cellular cytoplasm and into the cell in a more alkaline environment releases the proton resulting in a decrease of the intracellular pH (Canibe et al., 2001). This aspect influences the microbial metabolism, inhibiting the release of important enzymes and forcing the bacterial cell to use energy to release protons, leading to intracellular accumulation of anions and consequently reduces their growth rate and this due to energy consumption through the action of pumping ATPase proton pump (H+) until exhaustion of this bacterium (Gauthier, 2005).

These events reported above may have happened in our study because the total count of bacterial colonies was more favorable to treatment with PE and PE+A. As an example, the total colony count of treatment with PE was 70.9% lower than the control group, which brings us the possibility of PE effectively present antimicrobial properties. In this sense, the result for *Mac Conkey* method way helps to differentiate presumptively, the genera and species of microorganisms by staining or colony morphology. In our work, we found that the group receiving PE, had decreased by 91.78% in colony as compared to the control group. Through these results and the biochemical tests carried out, we can estimate that these colonies are of the genus *E. coli.* is the microorganism present in samples of feces and part of the intestinal saprophytic flora. *E. coli* is facultative anaerobic bacterium rod morphology, gram negative, fermenting lactose and easily grows in culture media such as *Mac Conkey* agar, forming large red colonies (Gyles and Fairbrother, 2004). Under biochemical evaluation, it shows a positive reaction for indole, negative for urease production and hydrogen sulfide and does not use citrate as a carbon source. These tests sallow a distinction between Enterobacteriaceae (Debroy and Maddox, 2001).

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Among the measures to reduce and control *E. coli* is antibiotic therapy (Glattleider, 1993). Sensitivity to antibiotics many work shaves been performed with varying results (Wilson, 1981; Brito et al., 2000). Because of the diversity of *E. coli* front antimicrobial behavior, especially by using sub doses of antibiotics and the easy transfer of resistance by plasmids from bacterial samples in our study was conducted antibiogram of feces samples from all treatments. We have observed that the treatments, actually did not influence the susceptibility testing results. The observed sensitivity can be the direct effect of antibiotic disks ad deed to the samples.

The antibiotics (Cefepine, Ceftriaxone, and Merepenem) were efficient in all samples. These antimicrobial agents are probably more efficient to inhibit microbial wall synthesis, resulting in bacterial death. This is possible because the Gram-negative cells such as *E. coli* have a much smaller amount of peptidoglycan than Gram positive. This makes its cell wall is not as thick and strong as the others above, but its structure is more complex due to the fact of the existence of membrane lipoproteins, polysaccharides and phospholipids, which involves its cell wall (Kinn et al., 2005).

To access the bacterial cell, antibiotics must cross through the cell wall porin protein channels embedded in lipid structure that present the inside with hydrophilic characteristics. So, antibiotics with greater activity against gram-negative are those with ionizable groups in its chemical structure (Guimarães et al., 2010).

Therefore, the characterization of an intestinal imbalance, diarrhea, commonly appears as an important sign of the complexity of the process. It is interesting to remember that bowel momentum is continuing, with immune responses of different intensities on substances or offensive and harmless agents. These tax challenges, often multifactorial actions that are caused by a plethora of causes, nutritional or dietary resources can be interesting to accelerate the recovery of any damage to the digestive system. To this end, further studies are needed to evaluate the optimal levels of inclusion and better combinations of plant extracts, given the possibility of improving the immune response of piglets in nursery phase.

CONCLUSION

The use of bioflavonoids and ascorbic acid did not alter the performance of pigs in the nursery phase. The use of plant extracts and associated with Amoxicillin reduces the count of bacterial colonies. It was observed that the high resistance of the studied samples for amoxicillin, neomycin and Norfloxacin. The antimicrobial Cefepine, Ceftriaxone and Merepenem were more efficient in inhibiting the growth of E. coli strains isolated from pigs supplemented with ascorbic acid and bioflavonoid.

DECLARATIONS

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Author's contribution

The authors, Magali Fernandes de Oliveira, Carlos Augusto Rigon Rossi, Matheus Shardong Lucca, Marcelo Soares, were responsible for collection and tabulation of data, experimental management of the pigs, as well as the article writing. The authors, Carlos Augusto Rigon Rossi, Vladimir de Oliveira, Julianni Dornelles, Luara Medianeira de Lima Schlösserand Cristian Guilherme Gräf were responsible for reviewing the manuscript.

Consent to publish

The authors, Magali Fernandes de Oliveira, Carlos Augusto Rigon Rossi, Matheus Shardong Lucca, Marcelo Soares, Vladimir de Oliveira, Julianni Dornelles, Luara Medianeira de Lima Schlösserand Cristian Guilherme Gräf are in favor of publishing the article entitled: "Performance and Microbiological Profiles of Piglets Fed with Diets Enriched with Bio-flavonoids and Ascorbic Acid" in the World's Veterinary Journal.

Competing interests

The authors have declared that there is no competing interest exists.

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Molecular Characterization of *Staphylococci* Isolated from Cattle with Mastitis

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ABSTRACT

This study was carried out in order to investigate the occurrence of some virulence genes of *Staphylococci* isolated from cattle with mastitis. A total number of 133 milk samples (45 from clinical mastitis and 88 from subclinical mastitis) were collected from dairy cattle in Kafr El-Sheikh and EL Gharbia Governorates, Egypt. The samples were examined for the presence of *Staphylococci* by classical bacteriological methods and were further characterized geno-typically. A total of 41 *Staphylococcus* isolates were recovered from cattle with mastitis with an incidence of 30.8%. Among the isolates, 21(15.8%) of *S. aureus* [6 from clinical mastitis (13.3%) and 15 from subclinical mastitis (17%)] and 20 (15%) isolates of CNS [8 from clinical mastitis (17.7%) and 12 from subclinical mastitis (13.6%)] were identified phenotypically. All isolates were screened for the detection of binding protein A (*spa*-X), haemolysine type A (*hla)*, Haemolysine type B (*hlb)*, and toxic shock syndrome (*tsst*-1*)* by PCR. The obtained results revealed that the *spa* Xgene was detected in all *Staphylococcus* isolates recovered from subclinical mastitis while in clinical mastitis was detected with an incidence of 42.9%. Haemolysine type A was detected in clinical and subclinical mastitis with an incidence of 71.4% and 70% respectively, while haemolysine type B was detected in clinical and subclinical mastitis with an incidence of 28.5% and 40% respectively. Toxic shock syndrome was not detected in any of the isolates. The data in the study provided an overview on the distribution of some virulence genes related to *Staphylococci* isolated from cattle with mastitis in Egypt.

Key words: Cattle, Mastitis, *Staphylococci*, Virulence gene, PCR

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INTRODUCTION

A wide variety of organisms have been identified as potential mastitis pathogens including *E. coli*, *S. uberis* and *S. aureus* (Radostitis, 2008; Erskine et al., 2002 and Gitau et al., 2003). Staphylococcal mastitis is a major concern in dairy farming and a serious source of subclinical and clinical Intra-Mammary Infections (IMI) in dairy cows leading to severe economic losses to the dairy industry worldwide (Momtaz et al., 2010; Atasever, 2012; Memon et al., 2013). Several epidemiological studies have suggested that *S. aureus* is the most prevalent in intramammary infections being related to more than 80% of the cases (Song et al., 2016). Recently published work has shown that 3 % of all animals are infected with *S. aureus* (Schukken et al., 2009), however, *S. aureus* represents 10 to 12 % of all clinical mastitis infections (Tenhagen et al., 2009).

Staphylococci have a capacity to produce a large number of putative virulence factors including surface-associated adhesins, a capsular polysaccharide, exo-enzymes, and exo-toxins. Some of these factors may be of more importance than others in different diseases or at different stages of the pathogenesis of particular infections, as not all factors are

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produced by each strain (Fitzgerald et al., 2000; Kalorey et al., 2007). One of the important virulence factors is staphylococcal exo-protein A (*spa*) which is a bacterial cell wall product that binds to the FC region of immunoglobulin G and impairs opsonisation by serum complement and phagocytosis by polymorphnuclear leukocytes (Alonso and Daggett, 2000; Eman et al., 2015). Therefore, low expression of protein A on the cell on surface of *S. aureus* resulted in greater number of free receptor sites for complement C3b and in increase in phagocytosis(Gao and Stewart, 2004).Staphylococcal hemolysins are identified as important virulence factors that contribute to bacterial invasion and escape from the host immune response (Rodrigues and da Silva, 2005). Alpha-hemolysin is the most studied and characterized *S. aureus* cytotoxin and is considered as a main pathogenic factor because of its hemolytic, dermonecrotic, and neurotoxic effects (Dinges et al., 2000). Additionally, beta hemolysin is a sphingomyelinase that is highly active against bovine erythrocytes (Larsen et al., 2002). The staphylococcal enterotoxins (SEs) are recognized agents of the staphylococcal food poisoning syndrome and may be involved in other types of infections with sequelae of shock in humans and animals (Bergdoll, 1981; Marrack and Kappler, 1990). A distantly related protein, toxic shock syndrome toxin-1 (*tsst*-1), also produced by *S. aureus*, was the first toxin shown to be involved in toxic shock syndrome, in both menstrual and non-menstrual cases (Bergdoll et al., 1981 and Schlievert et al., 1981). However, no immunological identity and little amino acid homology between *tsst*-1 and the staphylococcal enterotoxins exist (Blomster-Hautamaa et al., 1986).

The importance to evaluate *Staphylococcus* pathogenic activity assessing the combination of virulence genes has been emphasized both in human and in veterinary medicine (Zecconi et al., 2006; Piccinini et al., 2009). The genotype of *Staphylococcus* affects its prevalence and the number of infected quarters within a herd (Fournier et al., 2008). As information about the genetic variability of different *Staphylococcus* populations would help in the design of efficient therapeutic approaches and improvement of control measure. Few reports exist on the prevalence of *Staphylococci* among cattle with mastitis in Egypt. Consequently, the purpose of the present study was to investigate the prevalence and molecular characterization of *Staphylococci* isolated from dairy cattle with clinical and subclinical mastitis in Kafr El-Sheikh and EL Gharbia governorates, Egypt.

MATERIALS AND METHODS

Ethics committee approval

Ethical approval handlings of animals were according to the guidelines of animal ethics committee, faculty of veterinary medicine, Kafrelsheikh University, Egypt.

Sampling and bacterial isolation

A Total of 133 milk samples were collected aseptically from lactating cow (45 from cows with clinical mastitis and 88 from cows with sub clinical mastitis) in Kafr El-Sheikh and EL Gharbia Governorates, Egypt (Table 1). The samples were collected into sterile plastic tubes and submitted to the laboratory on ice packs as soon as possible for further bacteriological examinations. Samples were processed within 24-48 hours after reception. For subclinical mastitis, apparently normal milk samples were tested by using the California Mastitis Test (CMT), and were graded as negative, trace, weak, distinct, or strongly positive (Persson et al., 2011). Isolation of *Staphylococcus* was attempted from the CMT positive milk samples.

Milk samples were centrifuged; sediment was diluted with equal amount of sterile distilled water and streaked on Mannitol Salt Agar (Oxoid) at 37˚C for 48 h. Suspected colonies were selected and picked onto nutrient agar slants, all slants were incubated aerobically at 37˚C / 24 h for further identification. The isolates were identified as *S. aureus* based on their cultural, morphological and biochemical characteristics (tube coagulase, urease, sugar fermentation, catalase tests). Haemolytic activity was evaluated by plating suspected staphylococcal strains on plates of nutrient agar supplemented with 10% sterile sheep blood according to Quinn et al. (1994). Types of haemolysins were identified according to the lysis zone of each *Staphylococcus* isolates on the blood agar plate after 24 h incubation at 37˚C aerobically.

Table 1. Number and type of samples collected from cattle with mastitis

Farms	Clinical Samples	Subclinical samples	Total samples
Kafr El-Sheikh farms farms	30	61	91
El Gharbia farms	15	27	42
Total	45	88	133

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Molecular detection of *Staphylococci* virulence genes using PCR

DNA extraction. DNA extraction from samples was performed by using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56˚C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Molecular identification was conducted for the detection of the *S. aureus*16S rRNA gene by using species-specific primers described by Wada et al. (2010) and Pradhan et al. (2011). All *Staphylococcus* isolates were analyzed for four virulent genes including, *spa*X (X-region of protein A), *hla* gene (encoding α haemolysin), *hlb* gene (gene encoding β haemolysin) and *tsst*-1 gene (encoding toxic shock syndrome toxin). Several PCR protocols were used to detect the target genes of *Staphylococcus* isolates. PCR amplification was performed with PTC-100 programmable thermal cycler (Peltier Effect cycling, MJ, Research, INC, UK). DNA amplification was performed in a final reaction volume of 25 µl consisting of: 12.5µl of Emerald Amp GT PCR master mix (2X premix), 1 µl of 20 pmol of each primer, 6 µl of the DNA template and water, nuclease-free up to 25µl. In the present study, the primer pairs used in PCR protocols were selected from published papers based on specificity, compatibility and ability to target the genes of interest. The nucleotide sequences and anticipated molecular sizes of PCR amplified fragments for these gene-specific oligonucleotide primer sets are outlined in table 2. The cycling condition of each gene has been listed in table 3. After PCR reactions, the amplified products were separated by agarose gel electrophoresis (1.5% agarose gel containing 0.5 mg/ml ethidium bromide in 0.5 X Tris EDTA electrophoresis buffer) at 5V/ cm for 1.5 hand visualized under UV trans-luminator. A 100-bp DNA ladder (Fermentas, USA) was used as molecular weight marker.

Table 2. Target genes, primers sequences, and amplicon sizes of *Staphylococci* virulence determinants

Target genes	Primers sequences	Amplified segment (bp)	References
16S rRNA	TTCGTACCAGCCAGAGGTGGA	229	Wada et al. (2010)
	TCTTCAGCGCATCACCAATGCC		Pradhan et al. (2011)
spa (X region)	CAA GCA CCA AAA GAG GAA	226	Booth et al. (2001)
	CAC CAG GTT TAA CGA CAT		
hla	GGTTTA GCC TGG CCT TC	550	Akineden et al. (2001)
	CAT CAC GAA CTC GTT CG		Pradhan et al. (2011)
hlb	GCC AAA GCC GAA TCT AAG	850	Wada et al. (2010)
	CGC ATA TAC ATC CCA TGG C		Booth et al. (2001)
tsst-1	ATGGCAGCATCAGCTTGATA	350	Booth et al. (2001)
	TTTCCAATAACCACCCGTTT		
S. aureus nuc gene	GCGATTGATGGT GATACGGTT	267	Brakstad et al. (1992)
	AGCCAAGCCTTGACGAACTAA AGC		

Table 3. Cycling conditions of *Staphylococci* virulence determinants

Target gene	Primary denaturation	Amplification (35 cycles)			Final extension
		Secondary denaturation	Annealing	Extension	
16S rRNA	95˚C, 4 min.	95˚C, 45 sec.	50˚C, 60 sec.	72˚C, 30 sec.	72˚C, 10 min.
spa (X region)	94˚C, 4 min.	94˚C, 30 sec.	55˚C, 30 sec.	72˚C, 30 sec.	72˚C, 10 min.
hla	94˚C, 4 min.	94˚C, 45 sec.	50˚C, 45 sec.	72˚C, 60 sec.	72˚C, 10 min.
hlb	94˚C, 4 min.	94˚C, 45 sec.	57˚C, 60 sec.	72˚C, 80 sec.	72˚C, 10 min.
tsst-1	94˚C, 4 min.	94˚C, 2 min.	55 ˚C, 2 min	72˚C, 60 sec.	72˚C, 10 min.

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RESULTS

Bacteriological identification of *Staphylococcus* isolates

A total of 41 *Staphylococcus* isolates were recovered from the milk of 133 mastitic cattle in a prevalence rate of 30.8 %. Among the isolates, 21 were identified as *Staphylococcus aureus* based on cultural, morphological and biochemical characteristics in a prevalence rate of 15.8%, while the rest of the isolates (20 isolates) identified as Coagulase Negative *Staphylococci* (CNS) (15%). All the *S. aureus* isolates were positive for tube coagulase test; these strains were confirmed by PCR (Figure 1). Among the examined cattle, 6 *S. aureus* isolates were recovered from 45 cattles with clinical mastitis (13.3%) and 15 isolates were recovered from 88 cattles with subclinical mastitis (17 %). On the other hand, 8 and 12 isolates of CNS were isolated from cattle with clinical and subclinical mastitis in a prevalence rate of 17.7% and 13.6% respectively (Table 4).

Molecular detection of *Staphylococcus* virulence genes

Staphylococcus genus specific primers targeting 16S rRNA were employed for the specific confirmation of the *Staphylococcus* DNA. All the examined isolates yielded a specific single DNA band of 229 bp amplicon. Furthermore, a second confirmatory PCR for confirmation of atypical *S. aureus* was used (Figure 1). All *Staphylococcus* isolates were subjected to PCR for the detection of four virulent genes (*spa*X, *hla*, *hlb*and *tsst*-1). All of the isolates were found to be positive for one or more virulence-associated genes (Table 5). Among the examined 21 isolates of *S. aureus*, *spa*X gene was the predominant one, detected in 17 isolates with an incidence of 81 % (Figure 2).

*Spa*X gene was detected in all isolates of *S. aureus* recovered from subclinical mastitis (100%) while detected in 2 out of 6 isolates from clinical mastitis (33.3%). With regard to *hla,* it was detected from *S. aureus* isolated from clinical and subclinical mastitis with an incidence of 33.3% and 60% respectively (Figure 3). On the other hand, *hlb* gene was detected in *S. aureus* recovered from clinical mastitis and subclinical mastitis with an incidence of 16.6% and 46.6% respectively (Figure 4).

Regarding to incidence of virulent genes in CNS isolates, *spa* X gene was detected in all the isolates of *Staphylococcus* recovered from subclinical mastitis (100%) while in clinical mastitis, 4 out of 8isolates were positive (50%). Haemolysine type A was detected in all isolates of CNS from clinical mastitis (100%) while in subclinical mastitis, 5 isolates gave positive amplicons out of 12 (41.6%). With regard to *hlb* gene, it was detected in CNS isolates of clinical and subclinical mastitis with an incidence of 37.5% and 8.3%, respectively. All the isolates tested, failed to amplify *tsst*-1(Table 5).

Table 4. Incidence of *Staphylococci* in cattle with mastitis

Types of samples	No. of samples	*S. aureus*		CNS		Total isolates	
		No	%	No	%	No	%
Clinical mastitis	45	6	13.3	8	17.7	14	31.1
Subclinical mastitis	88	15	17	12	13.6	27	30.7
Total	133	21	15.8	20	15	41	30.8

Table 5. Distribution of virulence determinant genes in *Staphylococcus* isolates

Items	Clinical Mastitis						Subclinical mastitis					
	S. aureus (6*)		CNS (8)		Total (14)		*S. aureus* (15)		CNS (12)		Total (27)	
	No	%	No	%	No	%	No	%	No	%	No	%
***spa* (X region)**	2	33.3	4	50	6	42.9	15	100	12	100	27	100
hla	2	33.3	8	100	10	71.4	9	60	5	41.66	14	70
hlb	1	16.6	3	37.5	4	28.5	7	46.66	1	8.3	8	40
tsst-1	0	0	0	0	0	0	0	0	0	0	0	0

*Among the isolates, 21of *S. aureus* (15.8%) [6 from clinical mastitis (13.3%) and 15 from subclinical mastitis (17%)] and 20 isolates of CNS (15%) [8 from clinical mastitis (17.7%) and 12 from subclinical mastitis (13.6%)] were identified phenotypically.

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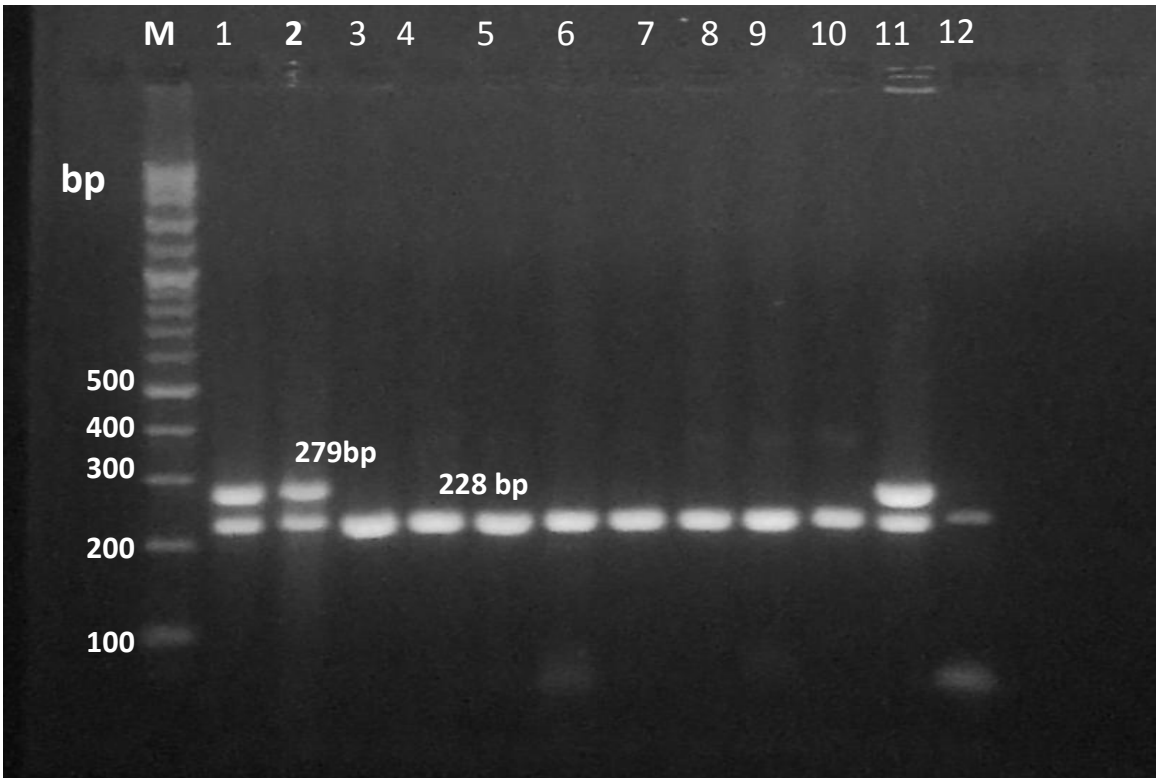

Figure 1. Agarose gel electrophoresis of duplex PCR amplification of 16S rRNA gene of *Staphylococci* (228 bp) and *S. aureus* specific *nuc* gene (267 bp). Lane M: 100 bp DNA ladder, Lane1, 2and 11: positive isolates for S. aureus. Lanes: 1-11 are positive isolates for genus Staphylococci (228 bp).

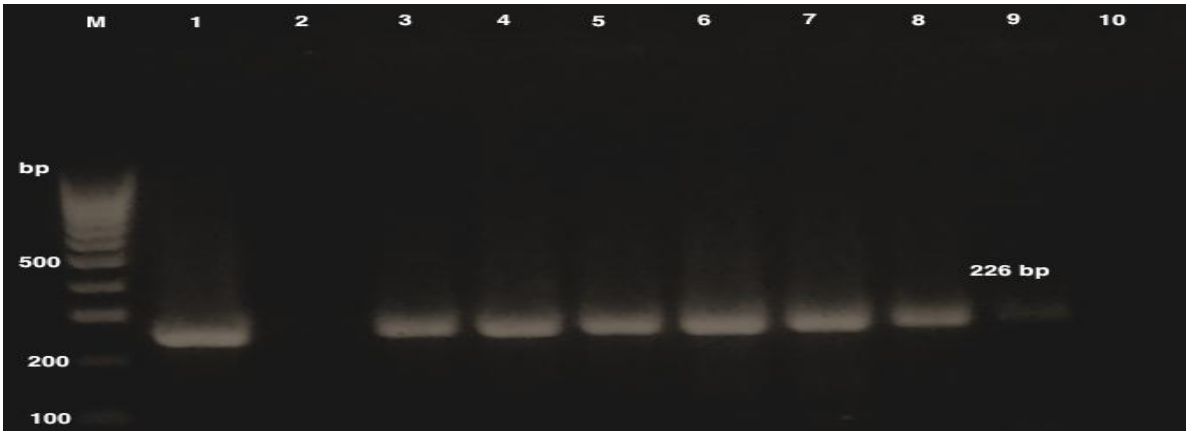

Figure 2. Agarose (1.5%) gel electrophoresis of *spa* X gene of *Staphylococcus* PCR products (226 bp). Lane M: 100 bp DNA markers, Lane 1, 3-8: positive samples, lanes 2 and 10: negative samples.

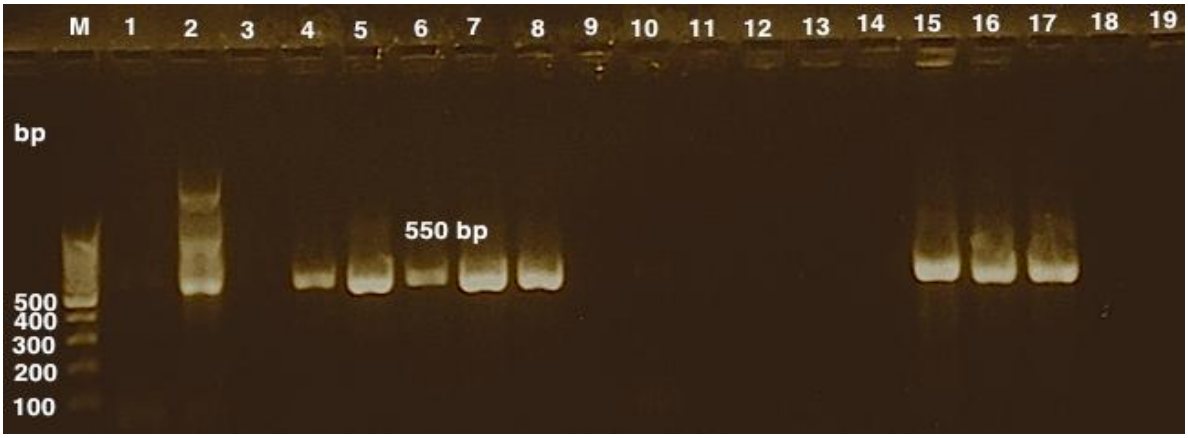

Figure 3. Agarose (1.5%) gel electrophoresis of *hla* gene of *Staphylococcus* PCR products (550 bp). Lane M: 100 bp DNA marker, Lanes: 1, 3, 9-14 and 18-19: negative isolates, lanes: 2, 4-8 and 15-17: positive isolates.

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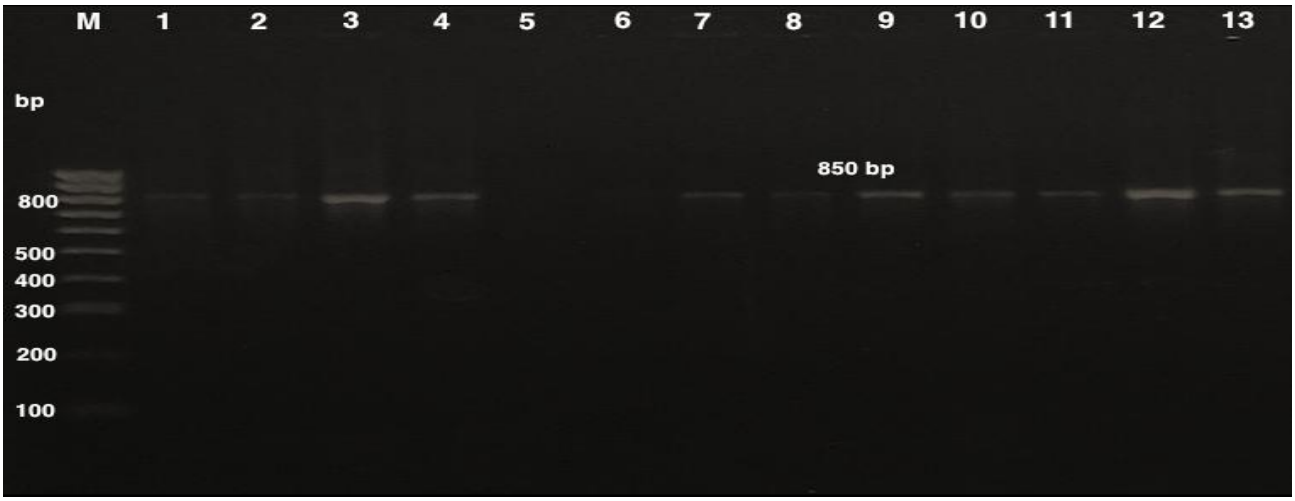

Figure 4. Agarose (1.5%) gel electrophoresis of *hlb* gene of *Staphylococcus* PCR products (850 bp). Lane M: 100 bp DNA marker, lanes 1-4 and 7-13: Positive isolates, lanes 5 and 6: negative isolates.

DISCUSSION

Cattle mastitis remains a serious and common disease in animals with significant economic losses in dairy industry worldwide (Momtaz et al., 2010; Atasever, 2012); therefore, knowing that mastitis causing bacteria and their virulent determinants using molecular methods is crucial to control the IMI (Ayman et al., 2015). A wide variety of organisms including *Staphylocooci*, *Streptococci*, *E.coli*, *Enterobacter spp.*, *Klebsiella spp.*, *Mycoplasma spp.* and *Corynebacterium* spp. are responsible for mastitis in animals. Among several bacterial pathogens that can cause mastitis, *Staphylococcus* species being the most lethal agent because it causes chronic and deep infection in the mammary glands that is extremely difficult to be cured (Momtaz et al., 2010).

The isolation of *Staphylococci* from milk alone is unequivocal in determining its role in the pathogenesis, therefore, *Staphylococci* virulent gene surveillance could be helpful in detecting genetic diversity among these major mastitis causing pathogens to develop effective control strategies against mastitis caused the pathogen (Khan et al., 2013).

In Egypt it was confirmed that *S.aureus* is considered as the predominant factor among mastitis causing pathogens followed by *S. agalactiae* (Elhaig and Selim, 2015) and *E.coli* (Hamed and Ziatoun, 2014). In the present study, out of 133 milk samples collected from cattle with mastitis, 41 *Staphylococcus* isolates were identified in a prevalence rate of 30.8%. This result was similar to the finding obtained by Barkema et al. (2009), however, a higher incidence rate was also recorded by Zecconi and Hahn (2000). Among the isolates, 21(51.2%) identified as *S. aureus* and 20 (48.78%) isolates as CNS based on biochemical tests and molecular identification.

In the current study, it was noticed that 15.7% (21/133) of *S. aureus* isolates were isolated and identified from cattle with mastitis. Similar results were reported by Botrel (2010), Nibret et al. (2011), Persson et al. (2011) and Hamid et al. (2017) who isolated *S. aureus* with an incidence of 15.8%, 16.5%, 19% and 22.5% respectively. However, higher detection rate was reported by other authors (Ahmed and Mohamed, 2009; Ashraf et al., 2016) who isolated *S. aureus* with an incidence of 77.1% and 52.5% respectively. Different PCR-based systems for the identification of *S. aureus* isolates from various origins have been used by numerous authors (Akineden et al., 2001; Nashev et al., 2004; Momtaz et al., 2010). As was found by Brakstad et al. (1992), the amplification of the gene encoding an *S. aureus*-specific part of the 16S rRNA revealed an amplicon with a size of 1,250 bp for all *S. aureus* isolates investigated.

The subclinical mastitis has special importance as it goes unnoticed and affects in a great extent the production's animal (Bhati et al., 2016). In subclinical mastitis, 15 isolates of *S. aureus* out of 88 samples were identified in a prevalence rate of 17%. Similar finding was reported by (Ak, 2000; Busato et al., 2000) who isolated *S.aureus* with a percentage of 13.3% and 16% respectively. In contrast to our data, several studies have demonstrated higher incidence of *S. aureus* in subclinical mastitis (Mokhbatly et al., 2001; Karimuribo et al., 2005; Khan and Muhammad, 2005; Ahmed and Mohamed, 2009; Alemu et al., 2014). In clinical mastitis, the incidence of *S. aureus* was 13.3% (6/45). This record agreed with the finding reported by Nevala et al. (2004), however, other studies have reported higher detection rates of *S. aureus* in clinical mastitis (Workineh et al., 2002; Elsayed et al., 2015). These variations are likely due to geographical area differences and time of sampling. In the last few years, the prevalence of CNS in mastitis was higher than those caused by *S. aureus*. In the present study, the prevalence of *S. aureus* and CNS in clinical mastitis was 13.3% and 17.7%

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respectively. Similar finding was also reported by Persson et al. (2011). In other studies, incidence of *S. aureus* was higher than that of CNS in clinical mastitis (Botrel et al., 2010; Rajeev et al., 2011; Eman et al., 2015).

Epidemiologic studies indicates that *S. aureus* strains agents of mastitis produce a group of virulence factors and it is believed that there is a relationship between the severity of mastitis and the virulence factors produced by *S. aureus* (Akineden et al., 2001). In the present study, molecular surveillance carried out in all isolates of *Staphylococci* to screen the presence of four putative virulence determinants encoding *spa* (the X-region of protein A) *hla* gene (encoding α haemolysin), *hlb* gene (gene encoding β haemolysin) and *tsst*-1 gene (encoding toxic shock syndrome toxin) by PCR. The distribution of virulent genes differed among the examined strains, some genes were present in all of the strains, but some genes were not found in any strain. The *spa*X gene typing in current study amplified (150-315bp) (Bhati et al., 2016). The genes *spa*X-region was detected in all isolates recovered from the samples of subclinical mastitis, it was consistent with the finding described by several authors (Coelho et al., 2011; Memon etal., 2013; Ashrafet al., 2016) that established the presence of *spa*X gene in nearly all of the isolates. Other reports in several countries including Italy (Dalla Pozza et al., 1999) India (Kumar et al., 2010) and Poland (Kahl et al., 2016), have previously identified this gene by 93-100% of *S. aureu s* isolated from subclinical form. The high incidence of *spa*X gene in *S. aureus* isolated from subclinical mastitis points to the potential role of this gene in this bacterium in a subclinical form, which unlike clinical mastitis, is milder and more difficult to detect.

Unlike the subclinical form, *spa*X was detected in *S. aureus* and CNS isolates in clinical mastitis with an incidence of 33.33% and 50% respectively. This is in contrast to the results described by (Stephan et al., 2001; Kalorey et al., 2007; Klein et al., 2012) who identified *spa*X in *Staphylococcus* isolates with an incidence of 76.5%, 70.3% and 85.9% respectively. In previous studies conducted by Salasia et al. (2011); Yang et al. (2012) and Wang et al. (2016), high frequency of *hla* was observed in *S. aureus* isolated from clinical mastitis (100%, 85%, and 94.3%, respectively). In our study, *hla* was observed in a percentage of 33.3%. However, *hla* was detected in all CNS isolates (100%). This finding might indicate the significant role of CNS isolates in the pathogenesis of bovine mastitis compared to *S. aureus* isolates. In subclinical mastitis, *hla* was detected in *S. aureus* and CNS with an incidence of (60%) and (41.66%) respectively, this result disagreed with Haveri et al. (2007); Salasia et al. (2011); Memon et al. (2013) they had recorded *hla*as 76%, 84% and 58% respectively. However; a higher frequency was recorded by Elsayed et al. (2015) and Ahmed et al. (2016). These different frequencies may be due to the different animal populations studied or the implemented methodologies, among other factors.

With regard to *hlb* in clinical mastitis, it was detected in *S. aureus* and CNS with an incidence of (16.66%) and (37.5%) respectively, these results correspond significantly with similar results obtained by Coelho et al. (2011). However, other investigators (Elsayed et al., 2015; Wang et al., 2016) have reported a relatively high incidence of this gene, while in subclinical mastitis, *hlb* was detected in *S. aureus* and CNS isolates with an incidence of (46.66%) and (8.3%) respectively, this percentage is higher than the one recorded by Coelho et al. (2011); Ahmed et al. (2016), however, in other studies, higher percentages of *hlb* in *S. aureus* isolates (Larsen et al., 2002, Salasia et al., 2011; Memon et al., 2013; Wang et al., 2016) were observed (97%, 84%, 71% and 79.1%, respectively). Toxic shock syndrome toxin (*tsst*-1) is one of the enterotoxogenic toxins responsible for food poisoning and is very important in the virulence of *Staphylococci*. In agreement with other studies (Nashev et al., 2004; Hassan et al., 2010; Gunaydin et al., 2011), we observed that the *tsst*-1 gene was not found in any isolate (0 %). However, these results are in disagreement with an earlier finding reported by Stephan et al. (2001) and Wang et al. (2016) that described higher *tsst*-1 gene positivity among *S. aureus* isolates (67.7% and 40% respectively).

CONCLUSION

Thus, in conclusion, the study provides a valuable insight into the virulence-associated genes of *Staphylococci*. The findings of this study indicated that all of the *Staphylococcus* isolates harbored one or more virulence-associated genes in dairy herds of cows suffering from clinical mastitis. The results also indicated that there is a direct relationship between the presence of *spa*X gene, it was the most frequent gene detected in examined isolates, and bovine mastitis especially subclinical type. Therefore, *spa* X gene could be considered as a good diagnostic method for typing of *Staphylococcus* isolates, which provided important results for the effective control of staphylococcal mastitis.

DECLARATIONS

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Author's contribution

Salwa M.Helmy and Ibrahim E. El Desouky planned and supervised the experiments and wrote the paper. Mohamed M. Ali and Hanaa A.Asfour performed the experiments and/or analyzed the data.

Competing of interest

The author declares that he has no conflict of interest with respect to the research, authorship, and/or publication of this article, the author declares that he has no competing interests.

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Parasitic Infection with Emphasis on *Tylodelphys* spp. as New Host and Locality Records in Nile Perch; *Lates niloticus* from Lake Nasser, Egypt

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ABSTRACT

A total number of 200 *Lates niloticus* were collected alive from several and various localities at Lake Nasser in Aswan governorate, to investigate the prevailing parasites that infect this fish species. All the examined fish were positive for one or more parasites, three trematodes of two families were identified: *Diplectanum simil*e, *Diplectanum lacustri*s and *Tylodelphys* spp. (recorded for the first time in *Lates niloticus* representing new host and locality records), two nematodes of two families: *Philometra ovata* and L_3 larvae of *Contracaecum* spp.(has zoonotic importance), one acanthocephalan parasite: *Rhadinorhynchus niloticus*, two crustaceans parasites of one family: *Ergasilus kandti* and *Ergasilus latus*, while no cestodal infections were recorded at all. The prevalence of trematodes was at 95% meanwhile the nematodes were at 100% in addition to the acanthocephalan parasite was at 24.5% as well, crustaceans parasites were at 69.5%. This study evaluated clinical signs, postmortem examinations, parasitological examinations, seasonal prevalence and histopathological investigations of infected fish in addition to the relation between fish age and parasitism was also described. This study builds on our current understanding of different parasites infecting the wild *Lates niloticus* and provides novel information on the patterns of the isolated parasites and also serves to reassure the consumers that the musculature (the edible part) of the fish was free from any parasitic infections and safe for human consumption provided that the fish must be eviscerated as soon as possible after being caught and adequately cooked.

Key words: *Lates niloticus*, Nile perch**,** *Tylodelphys* spp., *Philometra ovata*, Pathology, Lake Nasser

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INTRODUCTION

Fish is one of the most valuable sources of protein across the world as people obtain about 25% of their animal protein requirements from fish and shellfish (Matter et al., 2013). The rapid increase in the population in Egypt motivates the government to pay attention to the development of fisheries, especially in Lake Nasser. The production from such lake comprises a significant proportion of the Egyptian inland fisheries and is an important alternative source of protein in the face of increasing meat prices.

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In Lake Nasser, the predominant species for sale as fresh fish are the tilapias, followed by *Lates niloticus*, which constitute 25.07% of the fish in the lake (GAFRD, 2016). The annual production of this fish in 2010 was 12,311 tons, representing 0.9% of the total annual production of the fish wealth sector in Egypt (GAFRD, 2010). In Egypt, the Nile perch (*Lates niloticus*), commercially known as Samoos or Ishr-bayad, is a freshwater tropical, carnivorous fish of high commercial and recreational value in Africa, it inhabits a wide variety of habitats, including rivers, lakes, and irrigation channels (Aloo et al., 2017). *Lates niloticus* has been introduced to many lakes in Africa, including Lake Nasser where it is fished commercially. *Lates niloticus* has high edible white meat without bone rich in protein and vitamins especially omega-3 which is vital for human and considered a good fish for aquaculture development (Asnake, 2018).

In the last few years, the increase in parasitic infections among the fish of Lake Nasser has led to a drastic decrease in fish yields, low marketability, as well as rejection of the fish by consumers who fear the macroscopic parasites. Moreover, some parasitic infections in the fish have zoonotic importance, delayed sexual maturity of the fish and increased fish mortality causing great economic losses (Noga, 2010 and Younis et al., 2017).

Trematodes, cestodes, acanthocephalans and nematodes had contributed the most among the major parasitic groups found in freshwater fish (Schmidt, 1990). Migrating larvae of *Contracaecum and Philometra* may cause tissue damage (Noga, 2010). Copepods are extremely important in aquatic ecosystems, where they constitute a food source for small fish, an intermediate host for fish parasites or fish parasites themselves, and serve as vectors of disease (Piasecki, 2004).

Due to the scarcity of scientific information on the incidence, characteristics and effects of parasitic infection on *Lates niloticus* in Lake Nasser, the present work aims to make an- up- to- date study that identifies the present status of some parasitic diseases afflicting *Lates niloticus* at the lake, the seasonal incidence, the histopathological alterations in the infected fish and analyze the relationship between fish age and each detected parasite.

MATERIAL AND METHODS

Ethical approval

Animal ethics committee, Faculty of Fish and Fisheries Technology, Aswan University, Egypt, approved the protocol and conducting of the study.

Study area

The High Dam Lake was created as a result of the construction of the Aswan High Dam in the 1960's. The High Dam Lake extends for 480 kms, from the High Dam in Egypt to the Dal Cataract in Sudan, it lies300 km within the Egyptian borders (as Lake Nasser) and 180 km of it lies within the Sudanese borders (as Lake Nubia). Lake Nasser, together with Lake Nubia, is the second largest man-made lake in the world, after Lake Volta, in Ghana. Lake Nasser is now one of the most important sources of freshwater fish in Egypt.

Fish samples

A total number of 200 *Lates niloticus* of different weights and sizes (50 fish/ season) were collected, randomly and alive, from different localities of Lake Nasser at Aswan governorate during the period of February 2016 to January 2017. The collected fish were transferred alive and kept in prepared glass aquaria in the laboratory of fish diseases, Faculty of Fisheries and Fish Technology, Aswan University. Identification of the host and the length frequency measurements were taken by measuring the standard length (the measurement from the most anterior tip of the body to the posterior end of the vertebral column), the total weight was taken in grams, and scale samples relevant to age determination were collected for subsequent examination.

Age determination

Scales of each fish were extruded from the area below the lateral line at a level behind the pectoral fin on the left side, and used to determine age according to Adam (2004). As the age distribution was determined, an age-length key was constructed. From this key, the length distribution, the number of fish belonging to each age group was determined. All fish under study were belonging to age groups 1, 2, 3, 4, 5, 6, 7 and 8 years.

Clinical and postmortem examination

The fish were subjected to full clinical examination after that, they were euthanized rapidly by percussion stunning followed by destruction of the brain the procedures complied with local and national animal welfare laws, guidelines and policies, the external and internal gross lesions were recorded immediately according to the method described by Noga (2010).

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Parasitological examinations

Parasitological examinations were performed and then the collected parasites were washed, fixed, stained and permanently mounted according to the methods described by Lucky (1977) and Woodland (2006).

Identification of collected helminthes

The collected helminthes were identified according to the identification keys of Yamaguti (1958, 1961 and 1963); Thurston and Paperna (1969); Schaperclauus (1992) and Anderson (2000).

Histopathological examination

After necropsy, sections of gills, stomach, intestine, liver, and gas bladder were fixed immediately in 10% formalin and processed for histopathological evaluation, using the routine paraffin embedding method as described by Bancroft and Gamble (2007). Sections of 3µm thick were cut and stained using hematoxylin and eosin for light microscopic examination.

RESULTS

Population structure (age composition)

The frequency and age composition of *Lates niloticus* collected from the study area during the study period is presented in table 1. From the table, it is clear that age group 2 years old (55 %) of *Lates niloticus* was the most dominant group in the catch, followed by age group 1 (18.3 %), then age group 3 (17.8 %); the rest of the groups each comprise less than 8.9 %.

Table 1. Age–length key of *Lates niloticus* collected from lake Nasser, Aswan, Egypt showing frequency of fish specimens in different length groups and their distribution in each age group during the period of February 2016 to January 2017

Length groups (mm)	Freq.	Age groups							
		1	2	3	4	5	6	7	8
200	3	3	0	0	0	0	0	0	0
230	7	7	0	0	0	0	0	0	0
260	23	17	6	0	0	0	0	0	0
290	28	4	24	0	0	0	0	0	0
320	39	0	38	1	0	0	0	0	0
350	22	0	14	8	0	0	0	0	0
380	18	0	5	12	1	0	0	0	0
410	5	0	3	2	0	0	0	0	0
440	6	0	2	3	1	0	0	0	0
470	4	0	1	2	1	0	0	0	0
500	5	0	0	2	3	0	0	0	0
530	1	0	0	0	0	1	0	0	0
560	1	0	0	0	0	1	0	0	0
590	0	0	0	0	0	0	0	0	0
620	0	0	0	0	0	0	0	0	0
650	3	0	0	0	0	0	3	0	0
680	1	0	0	0	0	0	0	1	0
710	1	0	0	0	0	0	0	1	0
740	2	0	0	0	0	0	0	1	1
Sum	169	31	93	30	6	2	3	3	1

Freq. = frequency

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Clinical, postmortem and histopathological examinations induced by parasites

Most of the examined fish showed no pathognomonic abnormalities except heavy parasitic infestation. Moreover, the examined fish exhibited restlessness, poor appetite, emaciation, slight abdominal distension as well as respiratory difficulties manifested by surface swimming and gasping.

Emaciation, dark or pale body coloration, detachment of scales and fin erosions were noticed, especially in the pelvic and caudal areas, together with hemorrhagic spots on different parts of the fish's body and excessive mucus. Alternatively, paleness and congestion were observed in some parts of gills (Figure 1a and Figure 3a). Excessive mucous secretion and erosions of gills tips as well as white spots were observed (Figure 1a). Microscopically, gills revealed varying degrees of pathological changes including congestion of primary lamellae, interlamellar hyperplasia and adhesion of secondary lamellae, loss of the secondary lamellae in some sections (Figure 1b) and pillar cells hyperplasia at the tips of the primary lamellae (Figure 3d). Longitudinal sections of parasites including such crustaceans as *Ergasilus* spp., such as *Ergasilus latus* and *Ergasilus kandti* (Figures 1e and 1f), and monogenea spp. (Figure 3d) such as *Diplectanum simile* and *Diplectanum lacustris* were also observed.

The internal organs of naturally infected fish were pale and anemic, with enlargement and congestion of the spleen and liver (Figure 2a), together with congested and hemorrhagic gas bladder (Figure 4a), hemorrhage of the stomach as well as the enteritis was observed, especially in high parasitic load. Visible parasites could be seen by the naked eye (Figures 2a and 2b). The stomach and intestine revealed marked necrosis, sloughing of the covering epithelium and infiltrations of inflammatory cells, including mononuclear cells and heterophils in the mucosa and submucosa (Figures 6c, 6d, 6e and 6f). Moreover, encysted third stage larva of *Contracaecum* spp. was observed microscopically attached to the serosa of the stomach, together with the significant inflammation of its wall (Figure 2f). The gas bladder revealed hemorrhage and inflammation but the identified parasites, either nematode, or trematode, were not detected microscopically. The livers of the infected fish revealed marked congestion, fatty change and multifocal necrosis of the hepatocytes, which in some areas had infiltrated with mononuclear cells and heterophils (Figures 6a and 6b). Marked heterophils infiltrations were also observed. Proboscis of acanthocephalan was observed and surrounded with inflammatory cells including mononuclear cells and heterophils (Figure 5f).

Parasitological examinations

On the bases of the morphological examinations, the following parasites were identified from *Lates niloticus*: *Diplectanum simile* and *Diplectanum lacustris* (Figures 3b and 3c) as monogeneans trematodes microscopically exhibit a wide range of shapes and sizes as well as *Ergasilus kandti* and *Ergasilus latus* (Figures 1c and 1d) as crustacean parasites from the gills.

Tylodelphys spp., un-encysted metacercariae digenetic trematodes, used fish as intermediate host was recovered from the wall of the gas bladder and reported as new host and locality records. The live specimens were white in color and the stained one had linguiform body, measuring few millimeters with rounded anterior end and conical pointed posterior end (Figures 4b, 4c, 4d, 4e and 4f).

Philometra ovata as nematodes isolated from the gas bladder measuring few millimeters and the live specimens were white in color and transparent (Figures 4g and 4h).

Third stage larva of *Contracaecum* spp. nematodes encapsulated in fibrin sheath and attached to the alimentary canal and encysted in the wall of the stomach in heavy infestation. The liberated live larvae of *Contracaecum* spp. were reddish-yellow in color with long, cylindrical body measuring 25-55 milimeters with rounded anterior and tapered posterior ends (Figures 2b, 2c, 2d and 2e).

Rhadinorhynchus niloticus, acanthocephalan isolated from the stomach, intestine especially rectum and attached to any organ in the abdominal cavity when liberated from the stomach and intestine. It was white in color with long, cylindrical body, measuring 2-3 centimeters and had a slender, hollow construction proboscis that forms the anterior end (Figures 5a, 5b, 5c, 5d and 5e).

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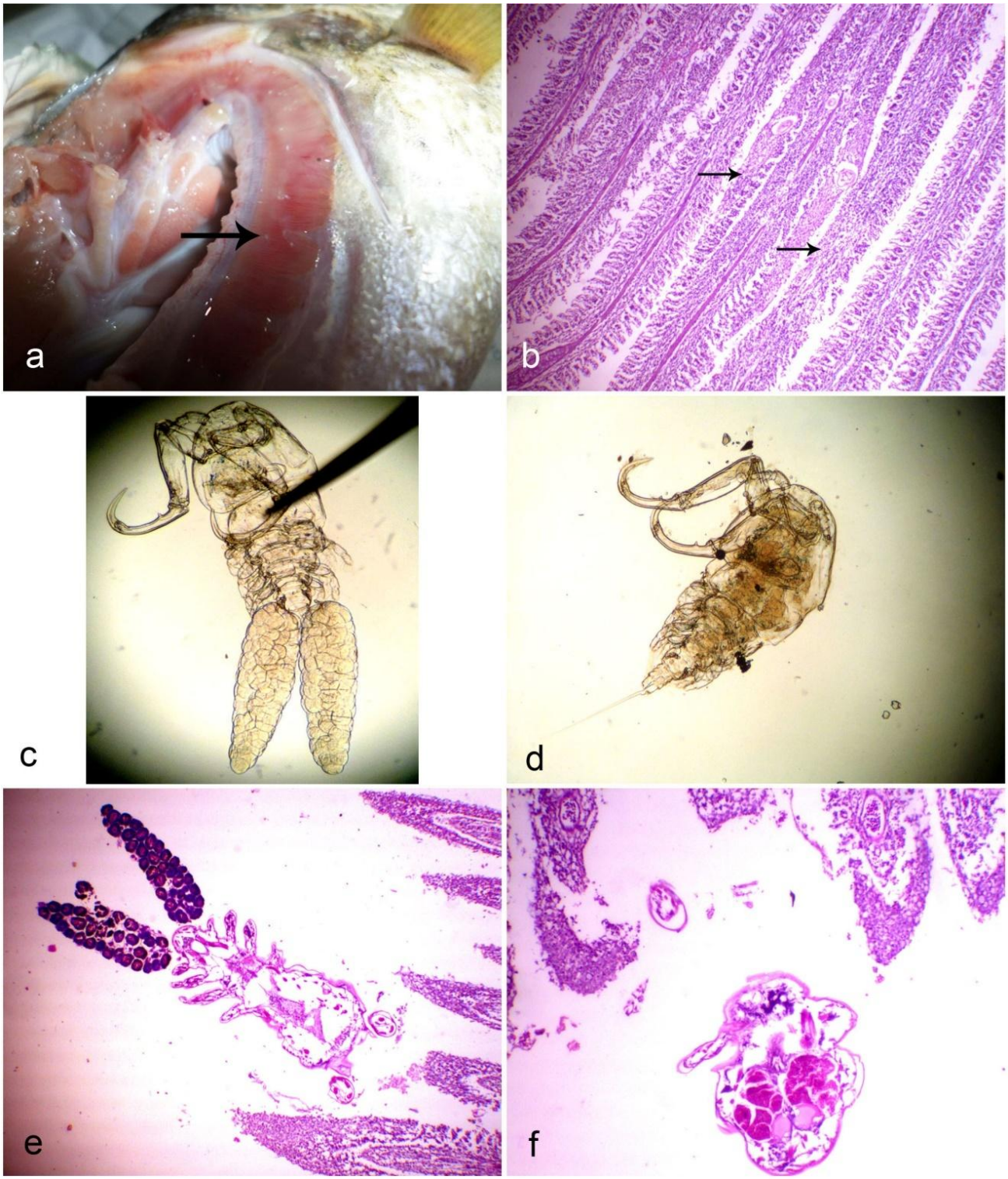

Figure 1. Gills of *Lates niloticus* from lake Nasser infected with *Ergasilus* spp. during the period of February 2016 to January 2017:

a. Pale gills of *Lates niloticus* showing excessive mucus and white dots (*Ergasilus* spp. -arrow).
b. Gills of *Lates niloticus* showing interlamellar hyperplasia and adhesion of secondary lamellae, loss of the secondary lamellae (arrow), H&E,× 100.
c. *Ergasilus latus* infecting the gills of *Lates niloticus*, × 240.
d. *Ergasilus kandti* infecting the gills of *Lates niloticus*, ×200.
e, f. Gills of *Lates niloticus* showing longitudinal sections of *Ergasilus latus* and *Ergasilus kandti* as well as pillar cells hyperplasia at the tips of the primary lamellae**,** H&E, × 200

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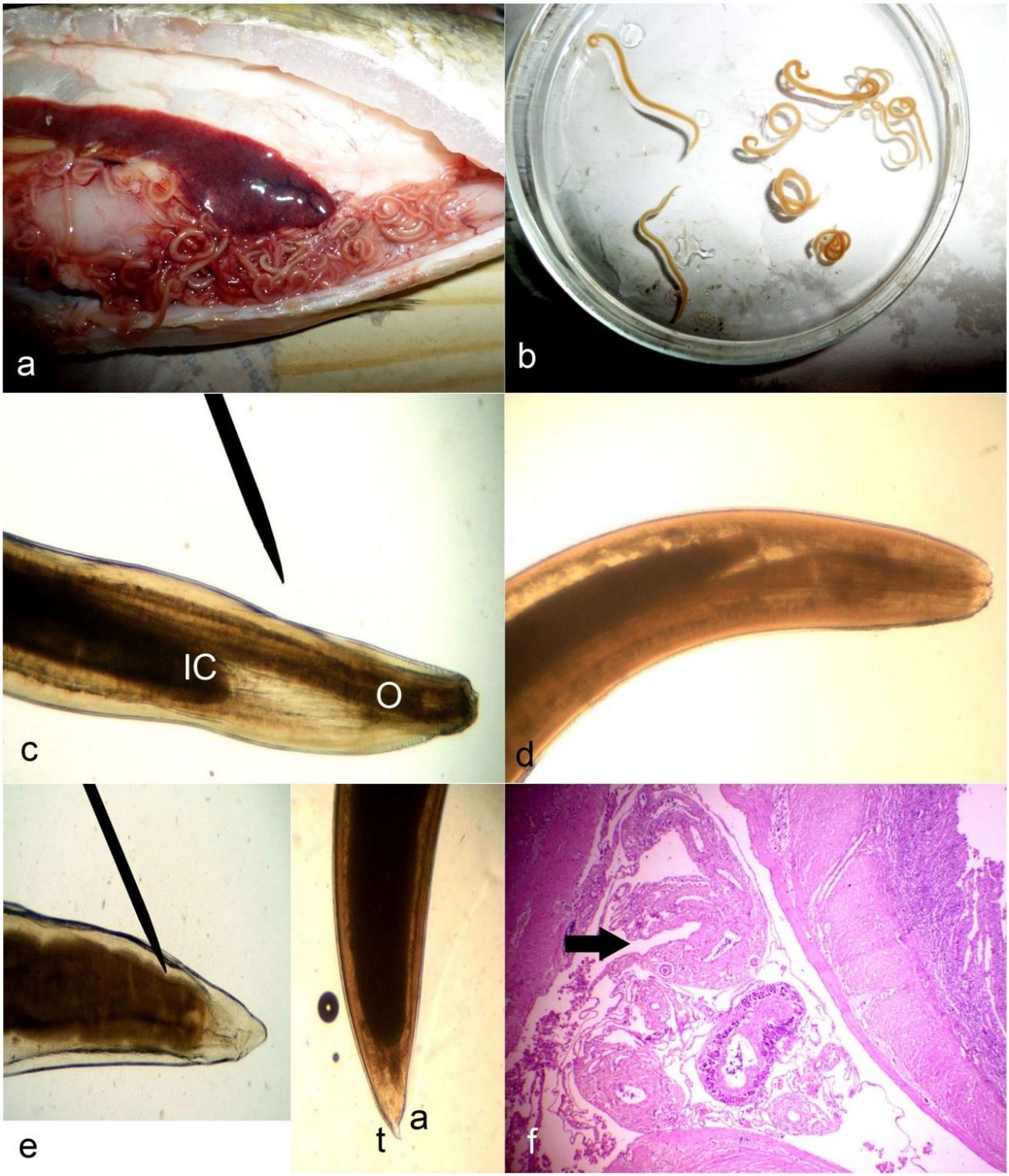

Figure 2. *Lates niloticus* from lake Nasser infected with *Contracaecum* spp. during the period of February 2016 to January 2017:

a. *Lates niloticus* showing congested liver, gas bladder and stomach as well as visible third stage larvae of *Contracaecum* spp. attached to the alimentary canal.
b. Liberated third stage larvae of *Contracaecum* spp. recovered from their fibrinous sheaths.
c. Anterior end of third stage larvae of *Contracaecum* spp. (O, oesphagous- IC, intestinal caecum),× 200.
d. Anterior end of third stage larvae of *Contracaecum* spp.,× 280.
e. Posterior end of third stage larvae of *Contracaecum* spp. (a, anal opening- t, tail end),× 200.
f. Stomach showing encysted third stage larva of *Contracaecum* spp. (arrow) as well as severe necrosis and infiltration of inflammatory cells in the lamina propria and submucosa, H&E,× 100

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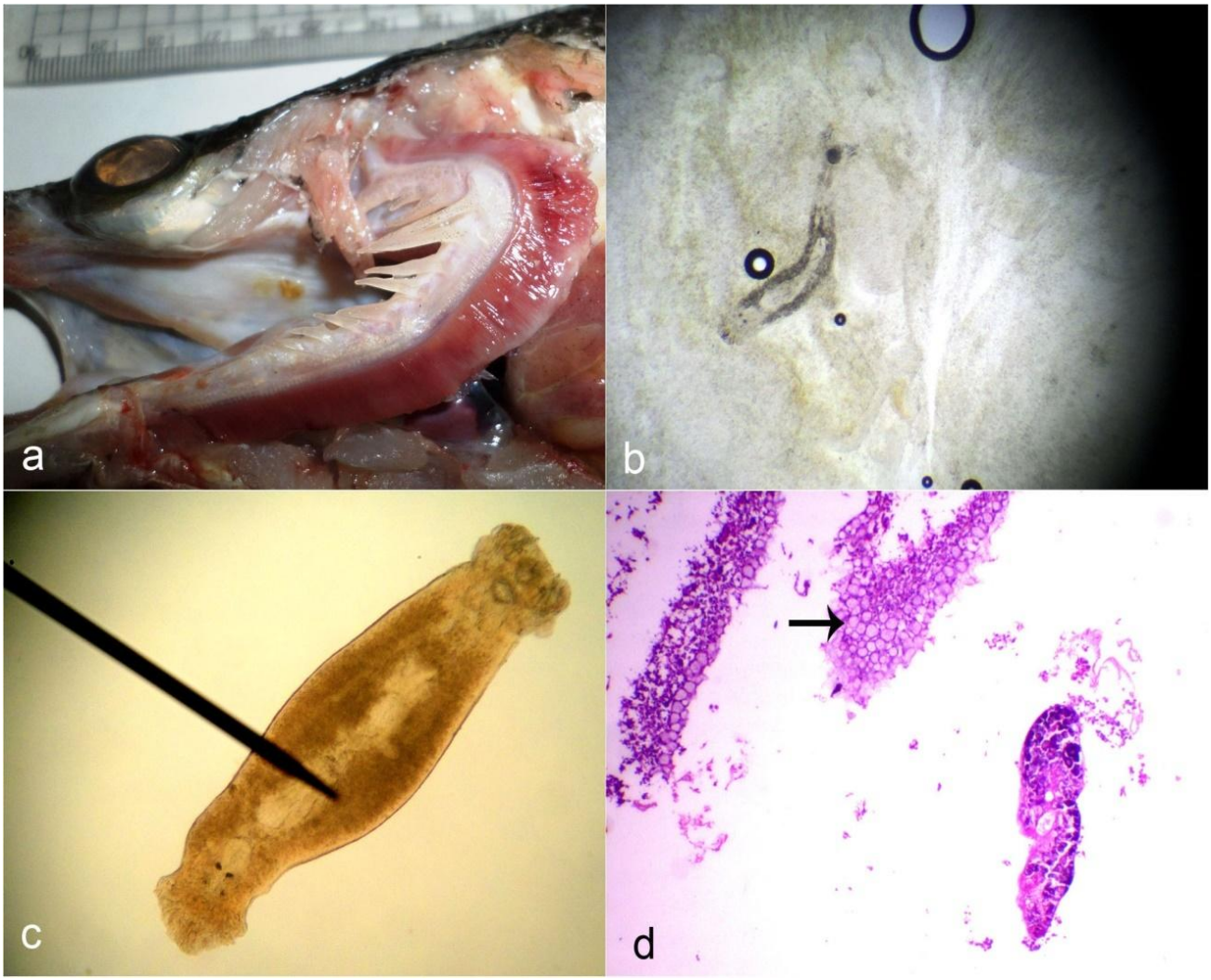

Figure 3. Gills of *Lates niloticus* from lake Nasser infected with monogenetic trematodes during the period of February 2016 to January 2017:

a. Gills of *Lates niloticus* infected with *Diplectanum* spp. showing excessive mucus, pale altered with congested areas as well as erosions of the gills tips.
b. Wet mount of *Diplectanum simile* infecting the gills of *Lates niloticus*,× 200.
c. *Diplectanum lacustris* infecting the gills of *Lates niloticus* stained with acetic acid alum carmine, ×500.
d. Gills of *Lates niloticus* showing pillar cells hyperplasia(arrow)at the tips of the primary lamellae as well as *monogenea* spp**.,** H&E,×200

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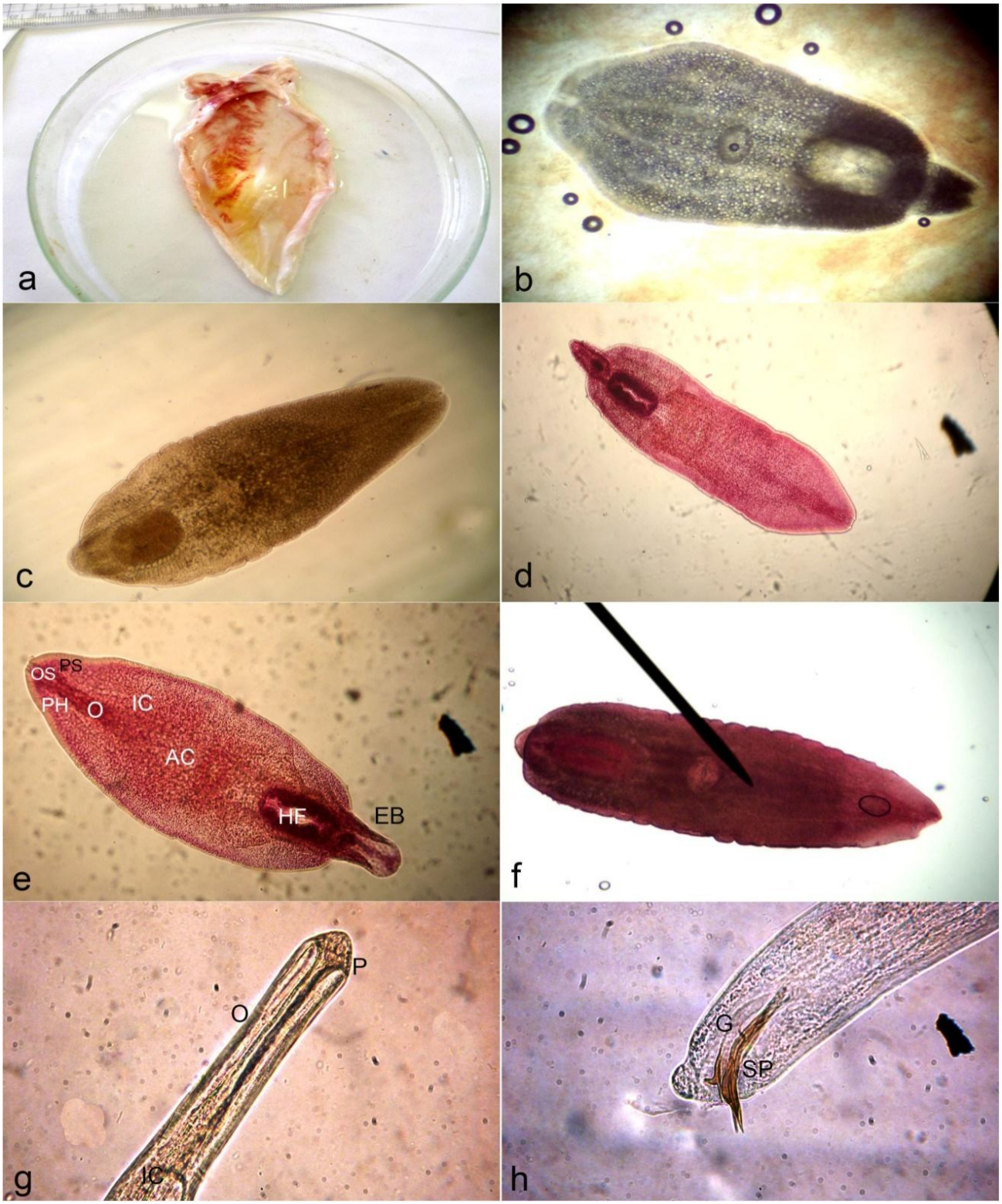

Figure 4. Gas bladder of *Lates niloticus* from lake Nasser infected with *Tylodelphys* spp. and *Philometra ovata* during the period of February 2016 to January 2017:

a. Gas bladder of *Lates niloticus* infected with *Tylodelphys* spp. and *Philometra ovata* showing slightly congested and hemorrhagic areas.

b. Wet mount of *Tylodelphys* spp. recovered from the gas bladder of *Lates niloticus*,× 200.

c,d,e,f. *Tylodelphys* spp. recovered from the gas bladder of *Lates niloticus* stained with acetic acid alum carmine,×200. (OS, oral sucker - PS, pseudosucker - PH, pharynx- O, oesophagus - IC, intestinal caeca - AC, acetabulum -EB, excretory bladder - HF, holdfast).

g. Anterior end of *Philometra ovata* recovered from the gas bladder of *Lates niloticus* (P, papillae - O, oesophagus - IC, intestine),× 4000.

h. Posterior end of male *Philometra ovata* (SP, spicules - G, gubernaculum) recovered from the gas bladder of *Lates niloticus*,× 4000

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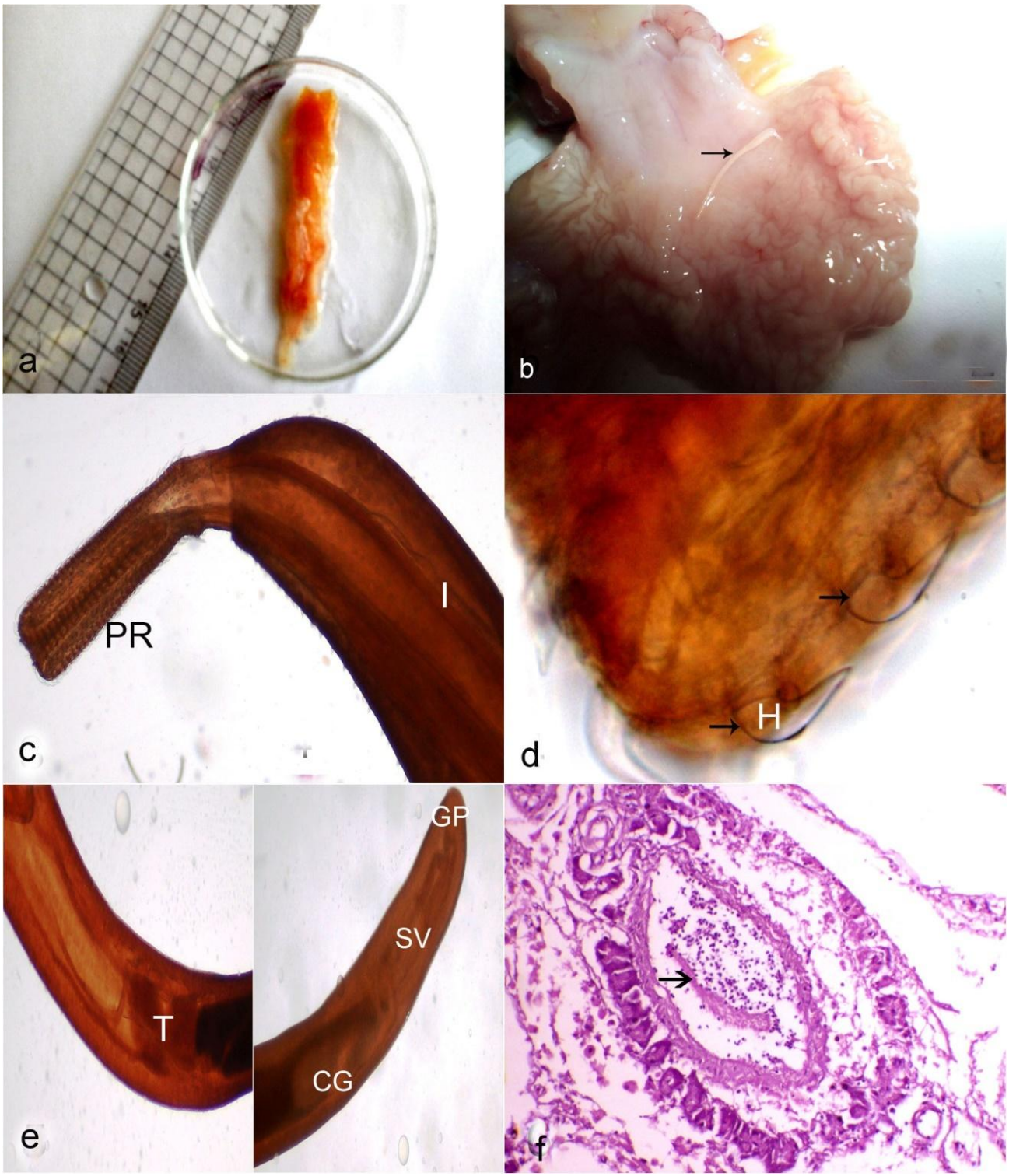

Figure 5. Rectum, stomach and liver of *Lates niloticus* from lake Nasser infected with *Rhadinorhynchus niloticus* during the period of February 2016 to January 2017:

a. *Rhadinorhynchus niloticus* inserted their proboscises in the congested rectum of *Lates niloticus*.
b. *Rhadinorhynchus niloticus* inserted its proboscis in the stomach of *Lates niloticus* (arrow).
c. Anterior end of *Rhadinorhynchus niloticus* infecting *Lates niloticus* (Pr, Proboscis - I, intestine),× 40.
d. Proboscis of *Rhadinorhynchus niloticus* infecting *Lates niloticus* showing the arranged hooks (H) (arrows),× 400.
e. Middle and posterior parts of *Rhadinorhynchus niloticus* infecting *Lates niloticus* (T, tests - CG, cement gland - SV, seminal vesicle - GP, genital pore),× 40.
f. Liver of *Lates niloticus* showing proboscis (arrow) of acanthocephalan surrounded with inflammatory cells including mononuclear cells and heterophils, H&E,× 200

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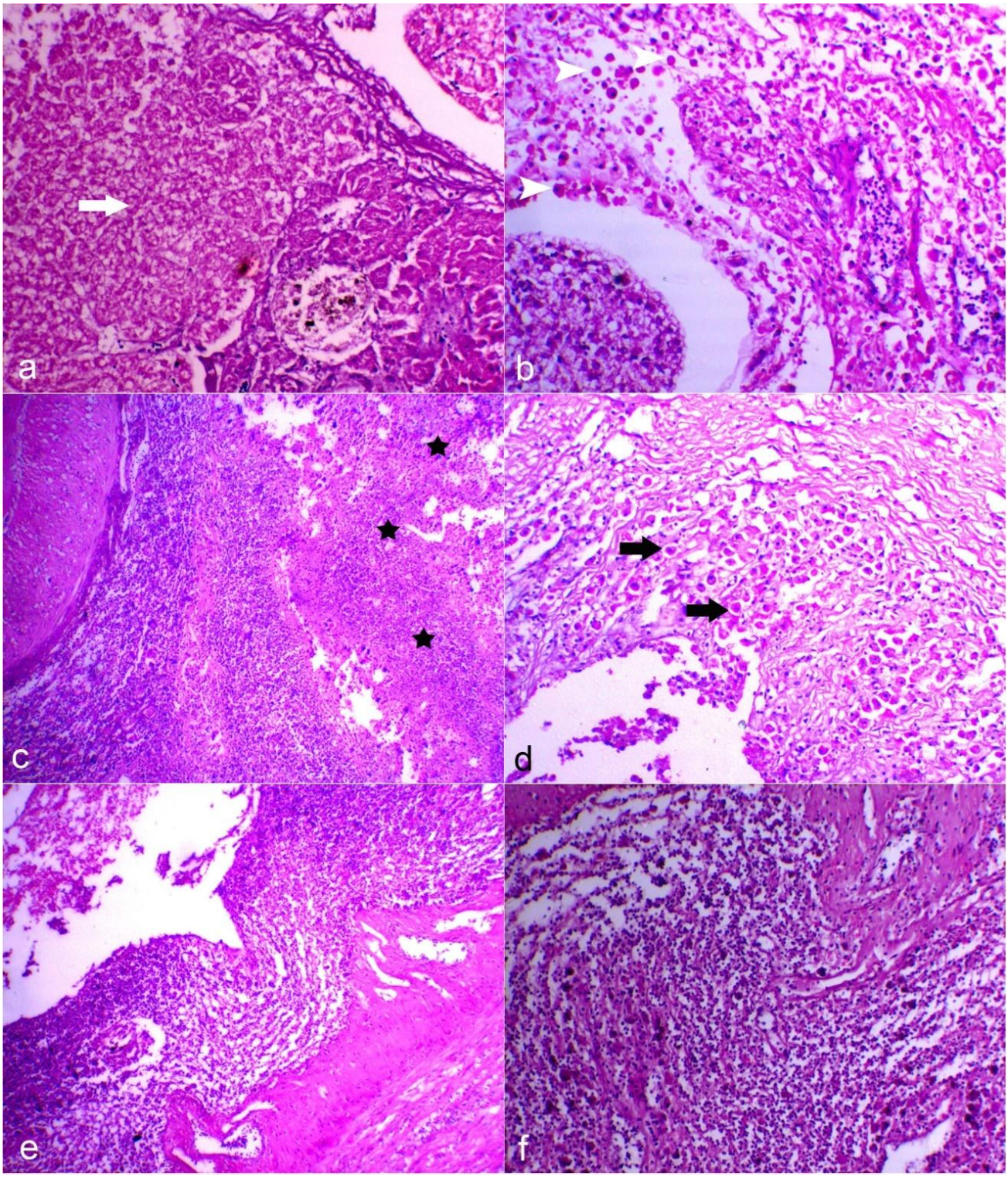

Figure 6. Liver, stomach and intestine of *Lates niloticus* from lake Nasser infected with *Contracaecum* spp. during the period of February 2016 to January 2017:

a. Liver showing marked necrosis of hepatocytes (arrow), H&E,× 100.

b. Liver showing fatty change and marked heterophils infiltrations (head arrow), H&E,× 200.

c. Stomach showing severe necrosis of the mucosa (asterisk) and infiltration of inflammatory cells in lamina propria and submucosa, H&E,× 100.

d. Higher magnification of Figure **6c**.
Stomach showing marked heterophils infiltrations (arrow) in the lamina propria, H&E,× 200.

e. Intestine showing severe necrosis of the mucosa and infiltration of inflammatory cells in the lamina propria and submucosa, H&E,× 100.

f. Higher magnification of Figure **6e**, intestine showing marked heterophils and mononuclear cells infiltrations in the lamina propria, H&E,× 200

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The total and the seasonal prevalence of detected parasites

The examined fish; *Lates niloticus* revealed a trematodal, nematodal, acanthocephalan and crustaceans infection rates of 95%, 100%, 24.5% and 69.5% respectively. However, there were no cestodal infections along the period of study, as presented in (Tables 2 and 3).

Concerning the seasonal dynamics of external *Diplectanum simile* and *Diplectanum lacustris* isolated from the gills of *Lates niloticus* with an overall prevalence of (30%), a higher infection rate was observed during autumn (50,58%), followed by summer (40,45%), spring (20,33%) and then winter (10, 18%) respectively, as presented in (Table 4). The incidence and intensity of infection with *Diplectanum* spp. in *Lates niloticus* was highest in the class 1 age group. *Tylodelphys* spp., un-encysted metacercaria isolated from the gas bladder, used *Lates niloticus* as an intermediate host with a total prevalence of (89%). A higher infection rate was observed during summer (96%), followed by autumn (90%), spring (86%) and then winter (84%), as described in (Table 4). The incidence and intensity of infection with *Tylodelphys* spp. in *Lates niloticus* increased with the class 2 age group.

Regarding the prevalence of different species of nematodal infections in *Lates niloticus*, *Philometra ovata* in the gas bladder was recorded with a total prevalence of 12.5 % and was higher in summer (20%) then spring (12%), winter (10%) and autumn (8%) (Table 4). It was observed much more in individuals belonging to the class 3 age group.

The third stage larvae of *Contracaecum* spp. in the abdominal cavity of the fish investigated were recorded with a prevalence rate of 100% throughout the period of study (Table 4). The recorded presence of *Rhadinorhynchus niloticus* isolated from the stomach and intestine of *Lates niloticus* was highest in winter (40%), then spring (30%), summer (20%) and autumn (8%) with a total prevalence of 24.5 % (Table 4).

The presence of nematodes (*Contracaecum* spp. and *Philometra ovata*) and the acanthocephalan (*Rhadinorhynchus niloticus*) in *Lates niloticus* had increased in incidence and intensity with the age of fish. *Ergasilus kandti* and *Ergasilus latus* were found in the gills of investigated fish at a higher infection rate in summer (90%, 92%), then spring (86%, 90%), autumn (82%, 87%) and winter (20%, 30%) respectively (Table 4). The incidence and intensity of infection with *Ergasilus* spp. in *Lates niloticus* was highest in the class 1 age.

In the present study, we noticed that while the gills are heavily infested with *Ergasilus* spp., the number of *Diplectanum* spp. drastically decreases or are even entirely absent. Interestingly, the musculature of *Lates niloticus* was completely free from any larvae, encysted meatacercariae and adult worms.

The parasites detected in *Lates niloticus* were found throughout the period of study. Some of them showed clear seasonal variation while the others were of nearly uniform frequency during the different seasons, varying only in the intensity of infections. The detected parasites were identified in their larval stages and adult worms were also reported.

DISCUSSION

Signs found on *Lates niloticus* infected with different parasites may be explained as sequences of parasites presenting different parts of the fish. Similar results have been reported on other fish hosts and other parasitic diseases (Hamouda, 2014). Few studies have reported on the occurrence of third stage larvae of *Contracaecum* spp. in *Lates niloticus* of Lake Nasser without any behavior abnormalities (Abd Alkareem, 2004).

Paleness and congestion in some parts of gills might be attributed to the mechanical injuries caused by *Diplectanum* spp. and *Ergasilus* spp. at the onset of infection leading to congestion. Indeed, when flukes increased in number and attacked the gills aggressively, they increase their feeding activities on the blood of the highly vascularized gills, causing anemia and pallor of the gills in the chronic phase. Excessive mucous secretion (as a defensive mechanism to diminish the irritant effect of the pathogen) and the white structures in the gills were *Ergasilus* spp. These results were nearly similar to those recorded by Noga (2010); Rashed (2013) and Hamouda (2014).

Internal gross lesions induced by parasites may be attributed to the presence of macroscopic parasites of third stage larvae of *Contracaecum* spp., *Philometra ovata*, *Tylodelphys* spp. and *Rhadinorhynchus niloticus* (which embed themselves in the lining mucosa of the stomach and intestine, causing local damage and possibly peritonitis). Moreover, proteolytic enzymes discharged from adult worms may be degrading the gastric and intestinal tissues (Woo, 1995). Helminthes produce toxic metabolic by-products which harm the infected host by causing occlusion of blood vessels, intestine and other ducts and resulting in the inflammation and congestion described in the internal organs. The above-mentioned reports suggesting the mechanism of tissue damage induced by parasites were confirmed histopathologically. However, no study has described in detail signs, lesions, evidences and histological damages induced by parasites in *Lates niloticus* as the present paper.

Latus niloticus is a carnivorous fish that assists in the greater transmission of parasites through feeding on young infested fish or aquatic animals that harbor it in the infective stages. This could be the reason for the maximum parasitic infestation rate (100%).

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There is scarcity of information on the isolated parasites of *Lates niloticus* from lake Nasser except 3rd stage larvae of *Contracaecum* spp. (Younis et al., 2017) and *Rhadinorhynchus niloticus*.

The metacercariae of *Tylodelphys* spp., are highly active and never encyst (Schaperclaus, 1992). Many authors around the world have detected *Tylodelphys* spp. in the vitreous humor of fish other than *Lates niloticus* (Otachi, 2009; Blasco-Costa et al., 2017 and Chaudhary et al., 2017) and from cranial cavity of *Clarias gariepinus* (Chibwana et al. 2015). But to our knowledge there is no record of isolation of such parasites from the swim bladder and from *Lates niloticus* so it is new host and locality records.

Philometra ovata was recorded with a total prevalence of 12.5 % and was highest in summer (20%) then spring (12%), winter (10%) and autumn (8%). It was observed much more in individuals belonging to the class 3 age group. These results were nearly identical with those recorded by Innal and Keskin (2005) on *Leucis cephalus* L. fish and lower than recorded by Y_I-T_E L (2014) on European minnows. Unidentified species of Philometroides were recorded from *Lates niloticus* in Egypt by El-Nafar et al. (1983) with a prevalence of 1.3%. *Philometra lati* isolated from the abdominal cavity of *Lates niloticus* and *Philometra spiriformis* sp. n. from capsules on the inner surface of the gill covers of *L. niloticus* were recorded by Moravec et al. (2009) in Kenya. Members of the family Philometridae has been recorded in different fish species other than *Lates niloticus* in Africa (Khalil, 1960, 1965, 1969 and 1973; Fahmy et al., 1976; El-Nafar et al., 1983 and Moravec and Van As, 2001).

The third stage larvae of *Contracaecum* spp. were recorded with a prevalence of 100% throughout the period of study. This result was identical with that recorded by Rabei (2009) and Younis et al. (2017) and this may be explained as the definitive hosts of *Contracaecum* spp. are pelicans, cormorants and herons and these are ubiquitous throughout the year around lake Nasser, so the infection was equally throughout the year. *Contracaecum* spp. larvae belonging to Anisakid nematodes which are considered to be responsible for anisakidosis disease, a serious zoonotic disease, and there has been a dramatic increase in its reported prevalence throughout the world in the last two decades (Lymbery and Cheah, 2007) so if the fish are not frozen or filleted soon after capture, larval nematode may migrate into the flesh. Accordingly, humans may be infected if viable larvae are consumed in uncooked or undercooked fishes (Younis et al., 2017).

Rhadinorhynchus niloticus was highest in winter (40%), then spring (30%), summer (20%) and autumn (8%) with a total prevalence of 24.5%. This was higher than that recorded by Ebraheem (1992) and Abd Elmageed (2015). The presence of nematodes (*Contracaecum* spp. and *Philometra ovata*) and the acanthocephalan (*Rhadinorhynchus niloticus*) in *Lates niloticus* increased in incidence and intensity with the age of fish and this may be owing to the accumulation of the worms over a longer period giving them available spaces for colonization and accumulation in addition to the larger size of fish, tend to be too big for the piscivorous bird to feed upon (Gichohi et al., 2008 and Zekarias and Yimer, 2008).

Ergasilus kandti and *Ergasilus latus* were found in the gills of investigated fish at a higher infection rate in summer (90, 92%), then spring (86, 90%), autumn (82, 87%) and winter (20, 30%), respectively. This was nearly similar to that recorded by Paperna (1968) but, Aladetohun et al. (2013) recorded the highest infestation rate of *Ergasilus* spp. in *Mugil cephalus* in the rainy seasons.

In the present study, we noticed that while the gills are heavily infested with *Ergasilus* spp., the number of *Diplectanum* spp. is drastically decreased or are even entirely absent. This may be because the heavy infestations with *Ergasilus* make the gills less suitable for monogeneans to establish themselves (Tiiurston and Paperna, 1969). *Ergasilus* feeding activity induces severe focal damage and very heavy infestations can be lethal (Noga, 2010).

The parasites detected in *Lates niloticus* were found throughout the period of study. Some of them showed clear seasonal variation while the others were of nearly uniform frequency during the different seasons, varying only in the intensity of infections. The snail is restricted to waters that remain warm (probably >17 ° C) year – round (Noga, 2010) and in Aswan, the high temperature in all seasons is maintained nearly throughout the year. This favors the development of snails, invertebrate hosts and the final hosts (fish, crocodiles, frogs, snakes and aquatic birds) as well as, an increase in plankton production, which is the source of nutrition of intermediate hosts.

The detected parasites were identified in both larval stages and adult worms, indicating that *Lates niloticus* act as intermediate, definitive or paratenic host contributing the expansion of infestations on intra or inter specific hosts. This could be due to the nature of the life cycle in these parasites, which involves the presence of snails or invertebrate (a crustacean; sometimes an annelid, coelenterate and mollusk) as first intermediate hosts, followed by fish as second intermediate host and finally aquatic birds, fish and reptiles as definitive final hosts (Syobodova and Kolarova, 2004).

Our results may differ partially or completely with many authors and this may be attributed to the different localities of the examined fish, type, age and sex of fish examined as well as, water hydrochemistry in each locality (Hamouda, 2014).

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Table 2. Prevalence of trematodes and nematodes among the examined *Lates niloticus* in lake Nasser, Egypt during the period of February 2016 to January 2017

Fish species	No. Exam. Fish	Trematodal infections										Nematodal infections					
		Diplectanum simile		*Diplectanum lacustris*		Total *Diplectanum* infections		*Tylodelphys* spp.		Total of trematodal infections		*Contracaecum* spp.		*Philometra ovata*		Total of nematodal infections	
		Infected No.	% of infections	Infected No.	% of infections	Infected No.	% of infections	Infected No.	% of infections	Infected No.	% of infections	Infected No.	% of infections	Infected No.	% of infections	Infected No.	% of infections
Lates niloticus	200	33	16.5	45	22.5	60	30	178	89	190	95	200	100	25	12.5	200	100

No. exam. = Number of examined

Table 3. Prevalence of acanthocephalans, crustaceans and cestodes among the examined *Lates niloticus* in lake Nasser, Egypt during the period of February 2016 to January 2017

Fish species	No. exam. Fish	*Rhadinorhynchus niloticus*		*Ergasilus* spp.						Cestodes	
				Ergasilus kandti		*Ergasilus latus*		Total Ergasilus infections			
		Infected No.	% of infections	Infected No.	% of infections	Infected No.	% of infections	Infected No.	% of infections	Infected No.	% of infections
Lates niloticus	200	49	24.5	60	30	90	45	139	69.5	0	0

No. exam. = Number of examined

Table 4. The seasonal prevalence, infection, intensity and mean/fish of helminth parasites in different organs of *Lates niloticus* from lake Nasser, Egypt during the period of February 2016 to January 2017

Type of parasite	% of infected fish	Seasonal prevalence				Susceptible organs	No. of Parasites per inf. fish	Mean /fish
		Spring	Summer	Autumn	Winter			
Diplectanum simile	16.5	20	40	50	10	Gills	4-10	4
Diplectanum lacustris	22.5	33	45	58	18	Gills	6-20	7
Tylodelphys spp.	89	86	96	90	84	Gas bladder	3-33	20
Philometra ovata	12.5	12	20	8	10	Gas bladder	6-60	28
Contracaecum L_3 larvae	100	100	100	100	100	Associated with the alimentary canal	1-99	33
Rhadinorhynchus niloticus	24.5	30	20	8	40	Stomach and intestine	1-7	4
Ergasilus kandti	30	86	90	82	20	Gills	10- 40	15
Ergasilus latus	45	90	92	87	23	Gill	10-60	22
Cestodes	0	0	0	0	0	——	0	0

No. = Number; inf. = infected

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CONCLUSION

Parasitic infestation of *Lates niloticus* is an important factor affecting wild populations of *Lates niloticus* and another aquatic species in Lake Nasser. Therefore, extended investigations about the effects of parasitic infection on fecundity, survival of this fish species are recommended to ensure successful fishery management plans.

Lates niloticus must be gutted soon after capture to avoid the attack of the detected parasites to its musculature which is completely free from any parasitic infection. The disposal of viscera or infected fish parts in water should be strenuously prohibited. Regular monitoring of the fish in Lake Nasser is a must. Further studies on *Tylodelphys* spp. in *Lates niloticus* (new host and locality records) are needed and urged.

DECLARATIONS

Author's contribution

All authors contributed equally to this work whereas they designed, conducted the research and wrote the manuscript.

Competing interests

The authors have declared that no competing interest exists.

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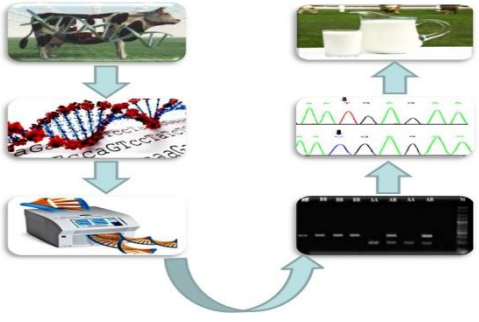

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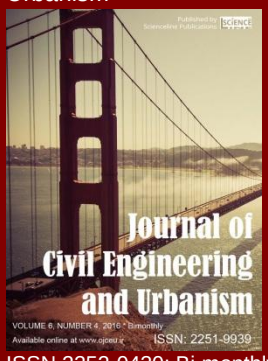

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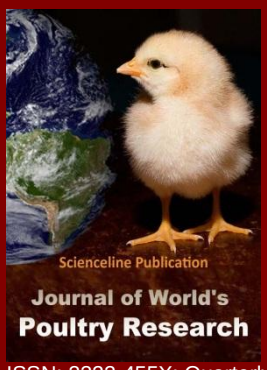

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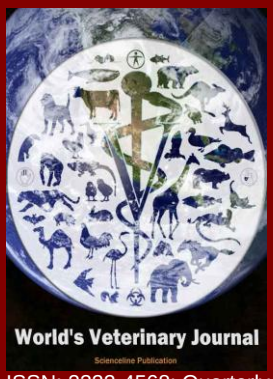

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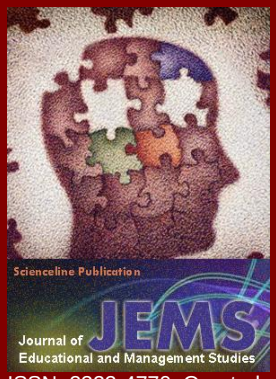

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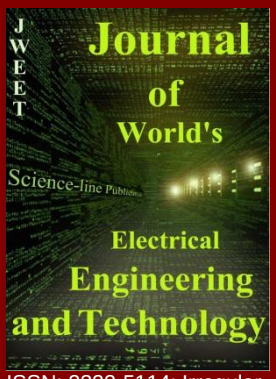

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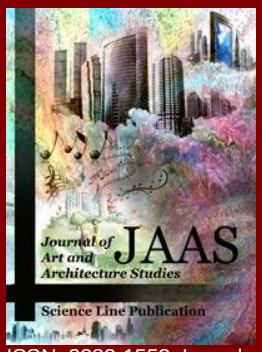

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