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Histopathological changes associated with *Mycoplasma* and some bacterial agents causing arthritis in ducks

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Abstract

Hundred leg samples were collected from fifty Pekin, Muscovy, and Mallard ducks (5 days to 2 years) suffering from arthritis at Sharkia governorate to be bacteriologically and pathologically examined. Specimens for bacteriological examinations included; the joint capsule, tendon and synovial fluid of knee, hock and tarsometatarsal joints. Pathological investigations include different specimens from the affected joints; parts of skin, synovium, tendon, cartilage of articular surfaces and epiphysis. The bacterial incidence rate including; *Staphylococcus* spp., *E. coli* spp., *M. synoviae*, *Salmonella* spp. and *Klebsiella* spp. was 70% of the collected samples. Single and mixed bacterial infections were obtained. The mixed infections represented 42% while the single ones represented only 28%. Molecular characterization using the specific gene of each bacterial agent revealing a specific DNA fragment for each one. Positive bacteriological samples were subjected to histopathological examination. Postmortem examinations revealed joints swelling, hyperkeratosis, erosions and ulcerations, while microscopic alterations of arthritic joints' parts revealed subdermal edema, epidermal leucocytic cells infiltrations, tendonitis, synovium fibrosis, erosions and ulcerations of articular surface cartilage with or without chondrocytes necrosis while joint epiphysis exhibited cyst formation and mostly calcification. Gentamicin, amoxicillin + clavulanic acid, erythromycin and colistin were the antibiotics of choice.

Keywords: *Ducks, arthritis, M. synoviae, bacterial agents, PCR, histopathological changes, in vitro antibiotics sensitivity.*

Introduction

Ducks production has been increased in a large scale, as the ducks rearing and management are usually easier in comparison with other poultry species as well as ducks are more resistance for diseases **(Krogdahl, 1985)**.

Mycoplasma gallisepticum (*MG*), *M. synoviae* (*MS*), *M. cloacale* (*MC*) and *M. anatis* were isolated from ducks kept in a yard in close contact with chickens that were infected with *MG*, *MS* and some other avian *Mycoplasma* species **(Bencia *et al*., 1988a).**

Although most poultry meat and eggs come from chickens, significant amounts of meat are produced from ducks and geese in certain parts of the world and they are considered of high nutritious value in addition to its delicious flavor. Duck and goose production accounts for about 7.5% of the total world poultry meat production **(Stipkovits and Szathmary, 2012)**.

Arthritis is one of the most common health issues of water fowls including duck and geese, although it was observed that some meat breed ducks are mostly affected earlier in life and

this is considered a great problem that needs rapid interference and proper treatment. Degenerative joint disease is known as osteoarthritis includes formation of osteophytes, degeneration or erosion of articular cartilage and fibrosis of joint capsules associated with inflammatory changes **(Rothschild and Ruhli, 2007).** Joint lesions are of multifactorial problem that has received extensive attention in the poultry industry **(McNamee *et al*., 1998).**

Bacterial arthritis in poultry commonly occurred after septicemia or localized infection to the joints is reported to be associated with many bacterial agents including *Staphylococcus, Mycoplasma, Escherichia, Erysipelothrix*, and *Listeria* **(Mohan *et al*., 2002)**. Arthritis is most commonly caused by *Staphylococcus aureus* **(McNamee and Smyth, 2000)** and sometimes involved *Escherichia coli* **(Chansiripornchai, 2009)** which is of veterinary importance in broiler breeders. It was found that domestic ducks with paratyphoid infections often have arthritis in hip and knee joints **(Friend, 1999).**

Bumble foot infection is characterized by planter pododermatitis, swelling and scabs at bottom of poultry feet which act as portal of entry for microorganisms, mostly *Staphylococcus* is involved in this kind of infection **(Ganguly and Wakchaurer, 2017)**.

Since the last era; arthritis is considered an economic disease causing condemnation of birds including ducks. Most publications on diseases of joints and related structures in poultry deal with inflammation of the synovial membranes and articular surfaces. A variety of bacterial species have been associated with joint infections in poultry. *Staphylococcus aureus* seems to be able to be localized in joints and tendon sheaths of most avian species **(Smart *et al*., 1968; Nairn and Watson, 1972**).

Studies of *Mycoplasma* infection in ducks goes back to the second part of 1960 when **Roberts (1964)** reported that in a case with mixed infections of influenza virus and mycoplasma, sinusitis was observed. Subsequently, more data about mycoplasma infections were published **(El-Ebeedy *et al*., 1987; Ivanics *et al*., 1988; Kempf, 1990; Lo *et al*., 1994; Abdul-Wahab *et al*., 1996)**. There are many data demonstrating that mycoplasma species are not always restricted to one animal species. Therefore it was not a surprise that mycoplasma of chicken origin have been cultured from ducks kept for several months on multiple-age chicken farms. In large studies **(Bencina *et al*., 1987a, b)** where 6 avian species was tested for mycoplasma, it was noted that *M. anatis* was found only in ducks but not in other avian species. *Mycoplasma anatis* together with other agents was associated with a nervous disease of ducks in a large farm **(Ivanics *et al*., 1988)**.

Swollen joints was the most prominent feature of septic arthritis which pathologically characterized by abscess foci either broken with deep ulcer and thickened skin or gangrenous dermatitis. Abscesses contained solidified cheesy-like material with ulcerative epithelial damage, marked hyperkeratosis and extensive dermal suppuration with presence of granular basophilic structures microscopically; probable to be bacterial colonies. Extensive fibrous connective tissue proliferation and dermal angiogenesis was also detected **Hassan *et al.* (2012)**. Multifocal degenerative changes in articular surfaces (fibrosis, metaplasia of cartilage and bone), enlarged joints, thick friable tissue around the joint and severe articular erosions, severe proliferation of fibrous connective tissue in/around articular surfaces, severe keratopathy with thick dull yellow exudate around the joints associated with mixed bacterial infection **(Degernes *et al*., 2011).**

Severe lymphocytes infiltrations, mild fibrinoheterophilic inflammation and thickened synovial membrane were observed due to hypertrophy and hyperplasia. Hyperemia and edema particularly at the margins of the articular cartilage were also detected. The synovial cavity was full of granulocytes, mononuclear cells, fibrin and cell debris associated with mycoplasma infection **(Kawakubo *et al.*, 1980)**.

Therefore this study aimed to throw spot of light on bacterial arthritis including *M. synoviae* in ducks that unfortunately hasn't received great attention and studying these infectious agents underlying this problem through molec-

ular diagnosis.

Materials and Methods

Samples:

Hundred leg samples were collected from fifty ducks of variable ages (5d- 2y) suffering arthritis and showing lameness from different farms at Sharkia governorate. Synovial fluid swabs, tendons, joint capsule and broken abscesses of knee, hock and foot pad joints of these ducks were submitted for isolation of mycoplasma and bacteriological isolation under complete aseptic conditions.

Isolation and biochemical identification of *M. synoviae*:

Samples were inoculated into sealed sterilized tubes containing 2-3ml of Frey's broth medium then incubated at 37ºC for 3-5 days and examined daily **(Kleven, 1997)**. Positive samples were cultured on Frey's agar medium for 2-3w in sealed container. The incubated plates were examined daily for the growth of the characteristic fried egg colonies by dissecting microscope. Further identification was carried out by digitonin sensitivity test **(Erno and Stipkovits, 1973 & Razin *et al.*, 1998).** Biochemical characterization of the isolated purified strains; glucose fermentation, arginine deamination and film & spot formation tests were done according to **Fabricant and Freundt (1967) &Watson *et al*. (1988).**

Isolation and biochemical identification of isolated bacteria:

Samples were enriched in nutrient broth at 37°C for 24h then inoculated into tryptic soya broth and Rappaport Vassiliadis (for *Salmonella* enrichment) then incubated at 37ºC for 24h and 42ºC for 18hr, respectively and then subcultured into Mannitol salt agar, paired parker media, MacConkey agar, Eosine Methylene agar, X.L.D. and S.S. agar, then incubated at 37ºC for 24-48hr. Suspected colonies from different media were picked up and subjected for further identification **(Quinn *et al*., 2011)**.

Bacterial colonies were identified morphologically using Gram's stain and biochemically by (IMVC tests, TSI, urea production, lysine decarboxylase tests). These tests were used to differentiate between members of *Enterobacteriaceae* according to **Quinn *et al*., (2011)**. Additionally, application of rapID ONE system for identification *Enterobacteriaceae* members (**Remel)**.

Coagulase test was performed on staphylyococci isolates to differentiate between coagulase positive staphylyococci and coagulase negative ones **(Markey *et al*., 2013).**

Genotypic identification of *MS* and other bacterial isolates:

DNA extraction:

M. synoviae: The genomic *M. synoviae* DNA was extracted using Patho Gen-spin DNA/ RNA extraction kit (Korea), Cat. No.17154, according to the manufacturers′ recommendations.

Other bacteria: DNA was extracted using QI-Aamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications according the manufacturers' recommendations.

Table (1). Primers used for molecular identification of *MS* and other bacterial agents

Species	Target gene	Primer sequence (5′→3′)	Amplicon Size (bp)	References
M. synoviae	*16SrRNA*	GAGAAGCAAAATAGTGATATCA CAGTCGTCTCCGAAGTTAACAA	214	**OIE, (2008)**
Staphylococcus	*16SrRNA*	CCTATAAGACTGGGATAACTTCGGG CTTTGAGTTTCAACCTTGCGGTCG	791	**Mason *et al.*, (2001)**
E. coli	*phoA*	CGATTCTGGAAATGGCAAAAG CGTGATCAGCGGTGACTATGAC	720	**Hu *et al.*, (2011)**
Klebsiella.	*gyrA*	CGC GTA CTA TAC GCC ATG AAC GTA ACC GTT GAT CAC TTC GGT CAG G	441	**Brisse and Verhoef, (2001)**
Salmonella	*invA*	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284	**Oliveira *et al.*, (2003)**

Table (2): PCR amplification and cycling protocol of*MS* and other bacterial isolates

Thermal profile	***16SrRNA* gene of *MS***	***16SrRNA* gene of *Staph.* spp.**	***phoA* gene of *E. coli* spp.**	***gyrA* gene of *Klebsiella* spp.**	***invA* gene of *Salmonella* spp.**
Initial denaturation		95˚C 5 min	94°C 5 min	95˚C 5 min	94˚C 5 min
Denaturation	94ºC 30 sec	94˚C 30 sec.	94°C 30 sec	94˚C 30 sec	94˚C 30 sec
Annealing	55ºC 30 sec	55˚C 40 sec	55°C 40 sec	55˚C 40 sec	55˚C 30 sec
Extension	72ºC 1 min	72˚C 45 sec	72°C 45 sec	72˚C 45 sec	72˚C 30 sec
Final extension	72ºC 5 min	72˚C 10 min	72°C 10 min	72˚C 10 min	72˚C 7 min
Amplification	40 cycles	35 cycles	35 cycles	35 cycles	35 cycles

PCR amplification:

16SrRNA gene of *MS* PCR amplifications were performed in Gradient Thermal cycler 1000S (Bio – RAD, USA). The reaction mixture (total volume of 50µl) was 25µl Dream green PCR Mix (Dream Taq Green PCR Master Mix (2X) Thermo Scientific Company, Lithuania), 5µl target DNA, 2µl of each primers (containing 10pmol/µl) and the mixture was completed by RNAseDNAse free sterile distilled water to 50µl.

Bacteria other than *MS* PCR amplifications were performed in a final volume of 25µl consisting of 12.5µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1µl of each primer of 20pmol concentration, 4.5µl of water, and 6µl of DNA template and nuclease-free water up to 25µl. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products:

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in TBE (1x)buffer. For gel analysis, 15µl of the products was loaded in each gel slot. Gelpilot 100bp and 100bp plus DNA ladders (Qiagen, Germany, GmbH) and a gene ruler 100bp ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software. Target genes, primers sequences and amplified product size were illustrated in **Tables 1 & 2**.

Antibacterial Sensitivity test:

The antibiotic susceptibility test was performed on the obtained isolates using agar disc diffusion method against different antibiotic discs representing different antibiotic classes **(Koneman *et al.*, 1997).** Bacterial cultures from different micro-organisms were prepared by inoculating colonies into Mueller Hinton broth then swabbed on Mueller Hinton agar (Oxoid) plates. Zones of inhibition were measured and results were interpreted according to

guidelines of **CLSI (2017).**

Pathological examination:
Tissue specimens:
Specimens in the form of several cut sections from the affected joints including different parts as; skin, tendons, cartilages and epiphysis were collected from sacrificed ducks suffering from arthritic joints then these specimens were fixed in 10% buffered neutral formalin. Paraffin sections of 5-7 micron thickness were prepared and stained with hematoxylin and eosin stain then examined microscopically **(Survarna *et al*., 2013)**.

Results and Discussion:
Locomotor disorders remain a challenge to the poultry industry, which represent not only a major economic concern but also a problem of poultry welfare **(Braga *et al*., 2016).**

Relatively little data in the literature have discussed degenerative joint disease in waterfowl especially that associated with infectious etiology, therefore; in the exiting study we discussed the role of *M. synovia* and other bacterial pathogens in arthritic ducks.

Recovery rate and biochemical identification of *MS* and other bacterial agents:
The isolation and identification results of bacterial agents causing arthritis in ducks using conventional methods depending on; their phenotypic characteristics on their specific media for each microbe. The recovery rate results revealed that 70% of collected samples (70/100) were positive for the bacteriological examinations. They were identified as *Staphylococcus* (32%) including; *S. aureus* (20%) and coagulase negative *Staphylococcus* (*CNS*) (12%), *Escherichia coli* (15%), *Mycoplasma synoviae* (12%), *Salmonella* spp. (7%) and *Klebsiella* spp. (4%).Concerning the mixed infections, it was observed in 42% of the positive examined samples as shown in **Table (3).** All *M. synoviae* isolates were digitonin sensitive, could ferment glucose and form film& spot but couldn't hydrolyze arginine **(Fabricant and Freundt, 1967)**.

Our results differed from those reported by **Bisgaard (1981)** in *Salmonella* spp*., S. aureus* and *E. coli* isolation rates from the joints of arthritic ducks. *S. typhimurium* recovery rate was 61% revealing that it was greatly higher than that of ours (7%). While the incidence rates of *S. aureus* and *E. coli* were18% and 6% respectively, which were lower than ours (20% and 15%, respectively).

The presence of mixed infection in arthritic joint samples was proved in previous studies as **Butterworth (1999)** who mentioned that *Salmonella* spp. associated with arthritis in ducks is found either as the sole bacterium genus or as part of a cocktail of bacteria as *S. aureus* and *E. coli***.**

On the contrary, these results disagreed with those studied by **Degernes *et al*. (2011)**. They failed to obtain any positive bacterial cultures from affected duck joints as they examined ducks with degenerative duck disease "DJD" with inflammatory changes on histopathology, but aerobic and mycoplasma microbiologic cultures collected post mortem were all negative and they supposed that if samples collected earlier in the clinical progression of disease it yields positive cultures, but unfortunately no ante-mortem microbiologic cultures were collected from clinically lame birds in this collection.

The obtained results were higher than that described by **Mamza *et al*., (2010)**, who isolated *E. coli* and *S. aureus* from the hock joints and digital pads of poultry with incidence of 4.2%, 8.1% from the hock joints and 14.3%, 10.7% from the digital pads, respectively.
Our results were lower than that reported by **Rasheed (2011)**, who isolated *S. aureus*, *E. coli*, and *S. saprophyticus* at percentage of 50.98%,and 7.8% each, respectively.
Bones, tendon sheaths and joints are the mostly frequent affected sites by *S. aureus* in poultry **(Adayel, 2005)** as this microbe had great affinity to collagen-rich surfaces such as the articular surface of joints and synovial sheaths located around the joints and tendons **(Rasheed, 2011).**
Wei *et al*. (1995) suggested that skin injury around the joint may play a very important role in the development of bacterial chondronecrosis or arthritis in poultry.

Table (3). Incidence rate of single and mixed bacterial agents in arthritic ducks

Bacterial agents	Single infection	Mixed infection	%	Total
S. aureus	5%	*S. aureus* + *M. synoviae*	7%	20%
		S. aureus + *E. coli*	4%	
		S. aureus + *Salmonella* spp.	2%	
		S. aureus + *CNS*	2%	
E. coli	7%	*E. coli* + *S. aureus*	4%	15%
		E. coli + *M. synoviae*	3%	
		E. coli + *Klebsiella* spp.	1%	
M. synoviae	0%	*M. synoviae* + *S. aureus*	7%	12%
		M. synoviae + *E. coli*	3%	
		M. synoviae + *CNS*	2%	
***CNS*spp.**	8%	*CNS* +*M. synoviae*	2%	12%
		CNS +*S.aureus*	2%	
***Salmonella*spp.**	5%	*Salmonella* spp.+ *S. aureus*	2%	7%
***Klebsiella*spp.**	3%	*Klebsiella* spp.+ *E. coli*	1%	4%

RapID ONE system:

Results of RapID ONE system raveled different 7 digits microcode and interrelated by ER-IC system 3137270, 3177270, 7137230, 63300210, 2300010, 0161001 and 4361011. Results were illustrated in **Fig. (1, 2 & 3)**.

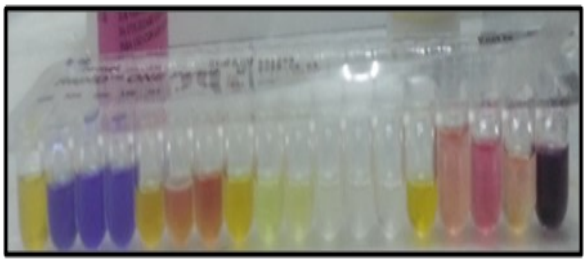

Fig. (1): Remel RapID ONE of *E. coli* isolate

Fig. (2): Remel RapID ONE of *K. pneumoniae* isolate

Fig. (3): Remel RapID ONE of *Salmonella* spp.

Molecular identification results:
Molecular genotyping is a promising technique in diagnosis of a wide range of micro-organisms. Polymerase chain reaction (PCR) test was applied to detect the specific genes for the different isolated bacterial agents causing arthritis in ducks. It is sensitive, specific, accurate and time saving test. These specific genes were *16SrRNA*gene for *M. synoviae* and *Staphylococcus, phoA* gene for *E. coli, gyrA* gene for *Klebsiella* and *invA* gene for *Salmonella* revealing characteristic DNA fragments at 214bp,791bp, 720bp, 441bp and 284bp, respectively **(OIE, 2008, Mason *et al.*, 2001, Hu *et al.*, 2011, Brisse and Verhoef, 2001and Oliveira *et al.*, 2003,** respectively**)**. These results were illustrated in **Fig. (4-8).**

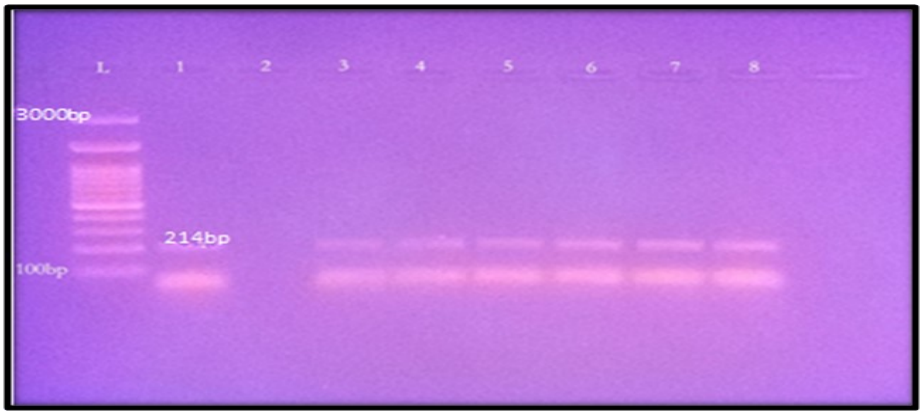

Fig. (4): Agrose gel electrophoresis of *16SrRNA* gene of *M. synoviae*
L: 100bp DNA marker
Lane 1: Positive control
Lane 2: Negative control
Lanes (3-8): Positive amplification for target gene at 214bp

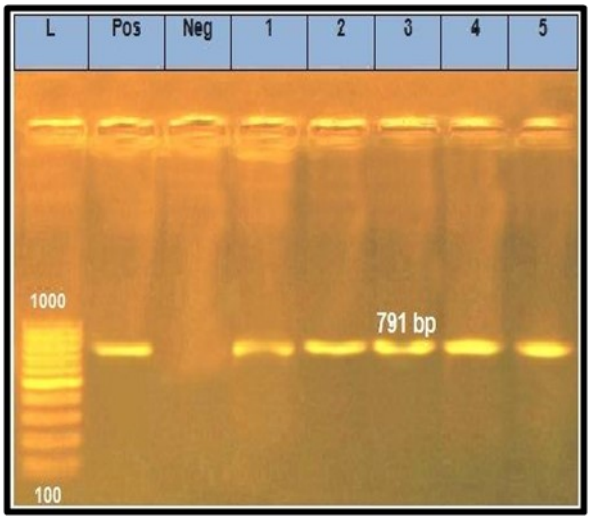

Fig. (5): Agrose gel electrophoresis of *16SrRNA* gene of *Staphylococcus* isolates
L: 100bp DNA marker
Pos.: Positive control
Neg.: Negative control
Lanes (1-5): Positive amplification for target gene at 791bp

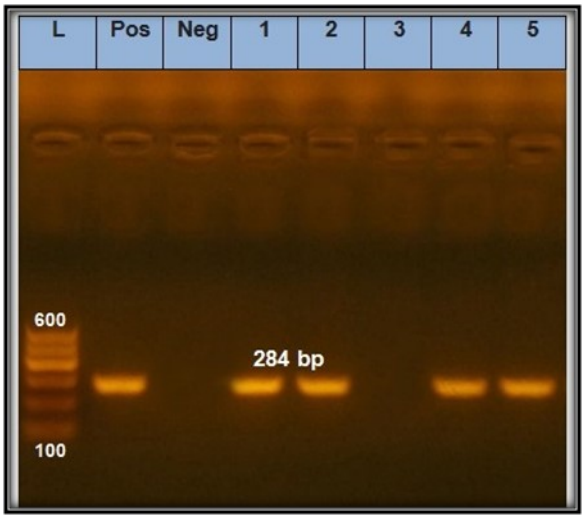

Fig. (6): Agrose gel electrophoresis of *invA* gene of *Salmonella* isolates
L: 100bp DNA marker
Pos.: Positive control
Neg.: Negative control
Lanes (1, 2, 4, 5): Positive amplification for target gene at 284bp

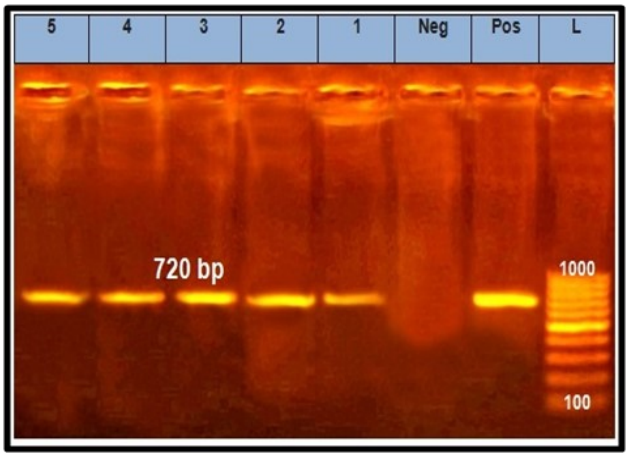

Fig. (7): Agrose gel electrophoresis of *phoA* gene of *E. coli* isolates
L: 100bp DNA marker
Pos.: Positive control
Neg.: Negative control
Lanes (1-5): positive amplification for target gene at 720bp

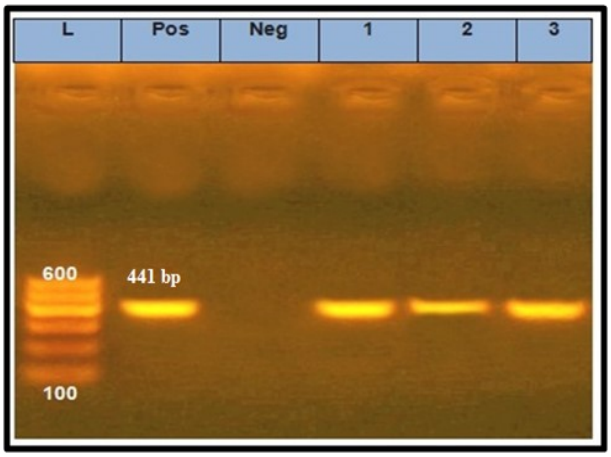

Fig. (8): Agrose gel electrophoresis of *gyrA* gene of *Klebsiella* isolates
L: 100bp DNA marker
Pos.: Positive control
Neg.: Negative control
Lanes (1-3): positive amplification for target gene at 441bp

Antibacterial susceptibility results:
The etiology and risk factors for degenerative joint disease in waterfowl is complicated and multi-factorial as in poultry, humans, and many other vertebrates **(Degernes *et al*., 2011).**

Duck Arthritis causes economic losses due to decreased feed intake, decreased feed conversion rate and thus condemnation. So, in-vitro antibiotic sensitivity test must be applied to obtain the antibiotic of choice for the treatment of different bacterial agents causing arthritis in ducks. Our results revealed relatively high level of antibiotic resistance among the examined isolates. Results were shown in **Table (4).**
In vitro sensitivity of *S. aureus* isolate to antibacterial agent was applied and isolates were mostly sensitive to amoxicillin +clavulanic acid, gentamicin, lincomycin and ciprofloxacin. As regard to antimicrobial suscep-tibility of *E. coli* isolates, they were sensitive to colistin, gentamycin and ciprofloxacin and were highly resistant to amoxicillin & clavulanic acid and this in accordance with **Eid *et al*. (2019)**.

In the medical control of *M. synoviae* infections the in vitro antibiotic susceptibility testing is crucial. Based on the in vitro examinations; erythromycin, lincomycin, doxycycline, and spectinomycin could be recommended for the therapy of *M. synoviae* infections **(Kreizinger *et al*., 2017).**

Mycoplasmas are resistant to β-lactam antimicrobials because of the lack of cell-wall and the bacteria are also resistant to membrane synthesis inhibitors **(Hannan, 2000).** *Mycoplasma* infected waterfowl and poultry flocks are usually treated with lincomycin and spectinomycin **(Stipkovits and Szathmary, 2012** and **Xiao *et al*., 2015)**

Salmonella isolates showed high sensitivity to ciprofloxacin, chloramphenicol and gentamycin whereas it exhibited high level of resistance to amoxicillin as mentioned by **Guo *et al*. (2019)** who detected that *Salmonella* isolated from the swollen joints of dead chickens revealed highest rate of resistance to ampicillin.
Klebsiella spp. showed resistance to most of the used antibiotics. Few isolates showed sensitivity to chloramphenicol and ciprofloxacin.

Table (4). In-vitro antibacterial sensitivity of *MS* and other bacterial isolates

Antibiotic	Symbol	Potency (µg)	Isolated bacterial agents									
			S. aureus N=20		*E. coli* N=15		*MS* N=12		*Salmonella* N=7		*Klebsiella* N=4	
			S	R	S	R	S	R	S	R	S	R
Amoxicillin+ clavulanic acid	AMC	30	14	6	1	14	-	-	1	6	0	4
Cefotaxime	CTX	30	5	15	7	8	-	-	4	3	0	4
Chloramphenicol	C	30	7	13	6	9	9	3	5	2	2	2
Ciprofloxacin	CIP	10	10	10	9	6	5	7	7	0	2	2
Colistin	CT	10	-	-	10	5	-	-	3	4	-	-
Doxycycline	DO	30	7	13	5	10	10	2	2	5	1	3
Erythromycin	E	15	8	12	0	15	12	0	0	7	0	4
Gentamycin	CN	30	13	7	10	5	7	5	5	2	1	3
Lincomycin	MY	15	11	9	-	-	11	1	-	-	-	-
Oxytetracycline	OT	30	-	-	-	-	9	3	-	-	-	-
Spectinomycin	SH	100	-	-	-	-	10	2	-	-	-	-
Sulfamethoxazole +trimethoprim	SXT	25	8	12	4	11	-	-	1	6	0	4

N= no. of positive isolates

Pathological Results:

Postmortem lesions:

Grossly, variable degrees of joints, foot pads and toes swellings were more pronounced mostly in old aged ducks. Most cases showed hyperkeratosis, erosions and/or ulceration of the articular surfaces. Other cases revealed necrosis with or without supportive arthritis so joints and tendon sheaths had viscid grey to yellow exudate. Synovium fibrosis became clear in some cases. Although tarso-metatarsal joints were the most affected ones in ducks, but the hock joint had clear affections with less frequently involved; this notice was similar to that obtained by **Degernes *et al.* (2011)**. Gross lesions associated with different isolated microbes were demonstrated in details in lesion score **(Table 5).** Some of these forms of injuries were demonstrated in **Fig. (9);** which were almost parallel to those obtained by **Kawakubo *et al.* (1980), Degernes *et al.* (2011) and Hassan *et al.* (2012).** Our macroscopic findings were relatively developed in mid and old aged ducks, while severe lesions were developed mostly in young ducks this result was in accordance with **Stipkovits and Szathmary (2012)** who proved that severe arthritis developed in about 30% of the 3-4w old birds at which the joints were swollen with accumulations of yellowish fluid in joints can be observed, from which *mycoplasma* can be cultured.

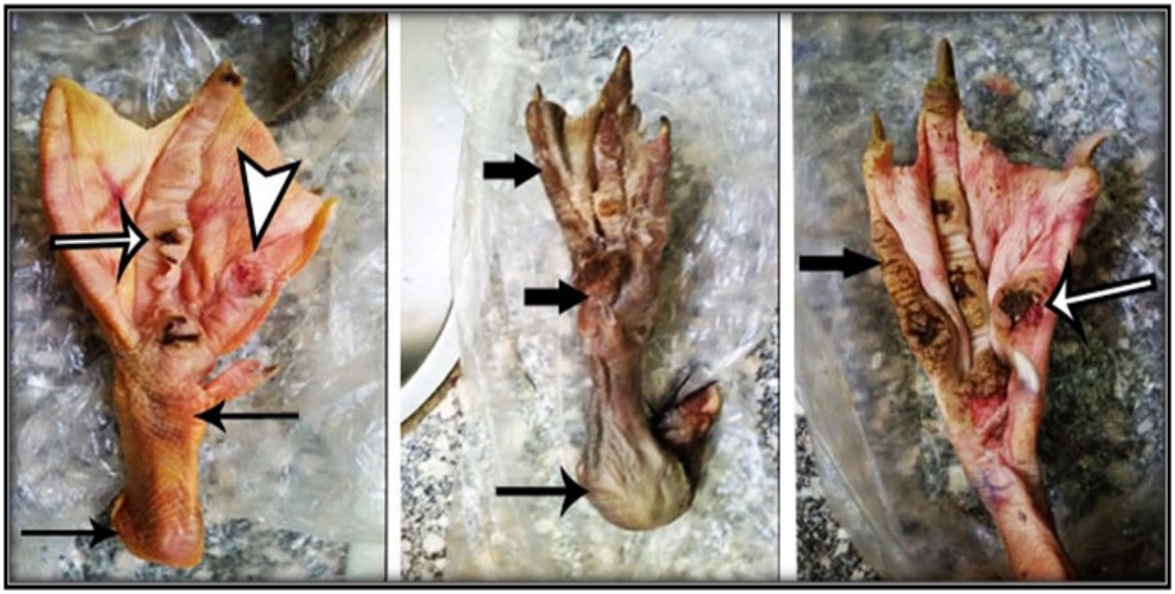

Fig. (9): Legs of (Pekin, Muscovy and Mallard) ducks suffering septic arthritis showing
Edematous joints **(thin black arrows)**
Dermal hyperkeratosis **(thick black arrows)**
Necrotic foci **(thin white arrows)**
Erosion and/or ulcer **(white headed arrows**)

Microscopic lesions:

Collected joint samples from sacrificed ducks suffering arthritis showed different shapes of skin affections. Hyperkeratosis of dermal surface was the most common lesion **(Fig. 10a)**, subdermal edema was frequently repeated in most examined joints **(Fig. 10b)**. Interstitial leucocytic cells infiltrates of the epidermal layer of skin was also observed **(Fig. 10c)**, while tendon showed moderate to severe congestion of blood vessels (tendonitis) **(Fig. 10d)**. Joint capsule showed fibrosis of the synovium **(Fig. 11a)** while, microscopic lesions of the articular cartilage ranged from focal or multifocal erosions to severe erosions and ulcers **(Fig. 11b)**, were noticed.

Articular cartilage showing necrosis of chondrocytes from its lacunae with or without focal erosions and ulcers **(Fig. 11c)**. Frequent affections were noticed in tarso-metatarsal joints and appeared as decalcified articular bones with interstitial deposition of basophilic material **(Fig. 11d)**. This material might be some bacterial colonies aggregations with tissues reaction matching with the same results and the explanations obtained by **Hassan *et al.* (2012)**. Cyst formation in the epiphysis center was also observed **(Fig. 12)**. Some of our results were in harmony with those obtained by **Degernes *et al.* (2011)** who noticed focal to severe erosions of the articular cartilage and cyst formation within the epiphysis with severe fibrosis of the joint capsule and synovium, he also described severe thickening of the joint capsule and synovium which was not matched with our results.

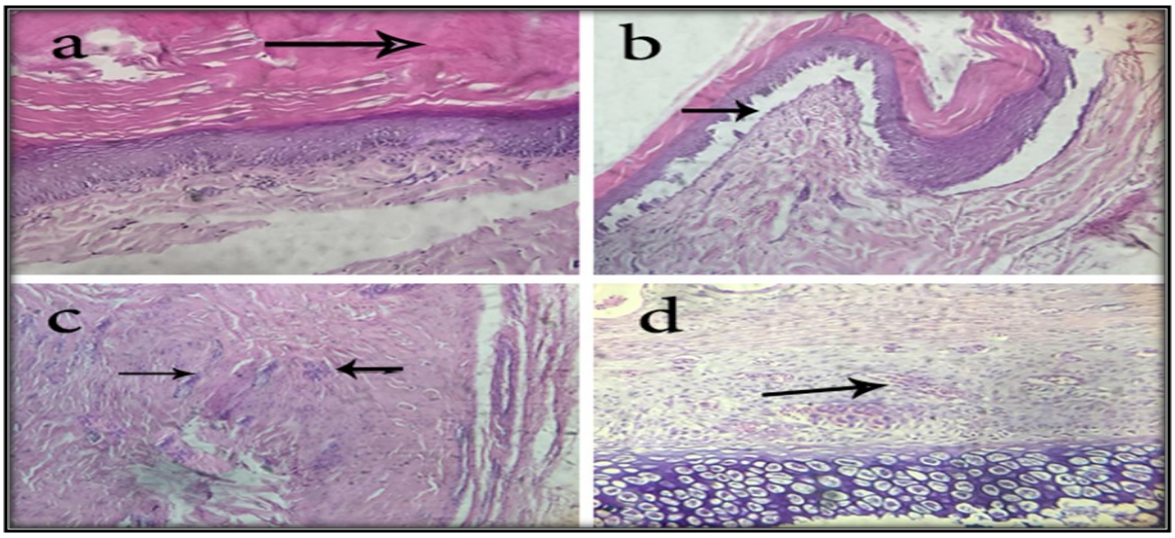

Fig. (10): Sacrificed (Pekin , Muscovy and Mallard) ducks joints suffering arthritis showing
(a): Photomicrograph of skin of3months aged Muscovy duck showing hyperkeratosis of dermal surface (arrow) **(H&E x100)**
(b): Photomicrograph of skin of 2months aged Pekin duck showing subdermal edema (thin arrow) **(H&E x100)**
(c): Photomicrograph of skin of 6months aged Muscovy duck showing interstitial leucocytic cells infiltrates the epidermal layer of skin (thin arrows) **(H&E x100)**
(d): Photomicrograph of tendon of 1year aged Mallard duck showing congestion of blood vessels (arrow) **(H&Ex100)**

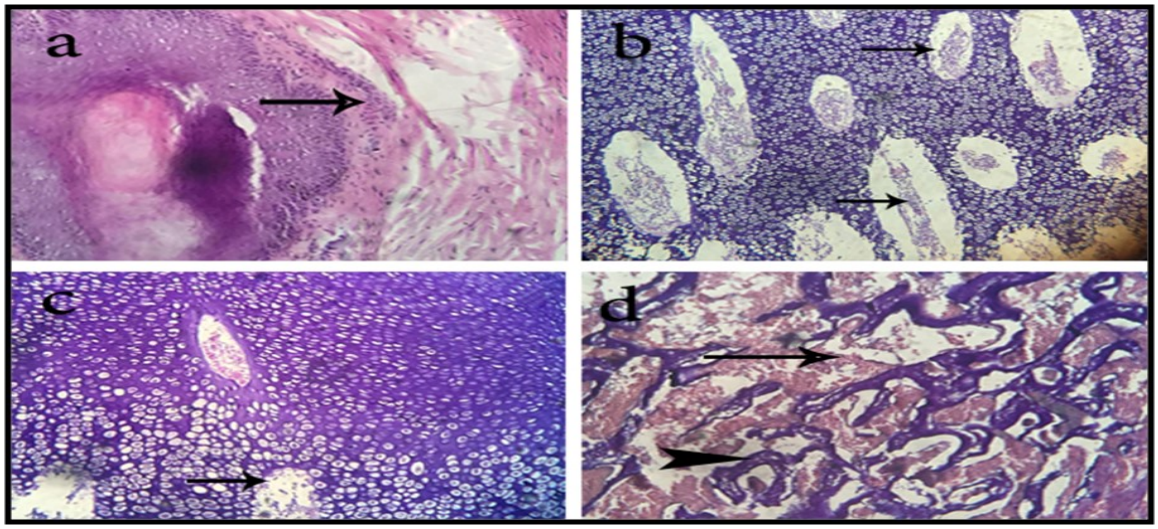

Fig. (11): Sacrificed (Pekin , Muscovy& Mallard) ducks joints suffering arthritis showing
(a): Photomicrograph of the joint capsule 7months aged Mallard duck showing fibrosis of the synovium (arrow) **(H&E x100)**
(b): Photomicrograph of the articular cartilage of 7months aged Muscovy duck showing multifocal erosions and ulcers (arrow) **(H&E x100)**
(c): Photomicrograph of the articular cartilage of 1 and half year Pekin duck showing necrosis of chondrocyte from its lacunae with focal erosion and ulcers (arrow) **(H&E x100)**
(d): Photomicrograph of tarso-metatarsal joint of 3months aged Muscovy duck showing decalcification of articular bone (arrow) with deposition of basophilic material (head arrow) **(H&E x100)**

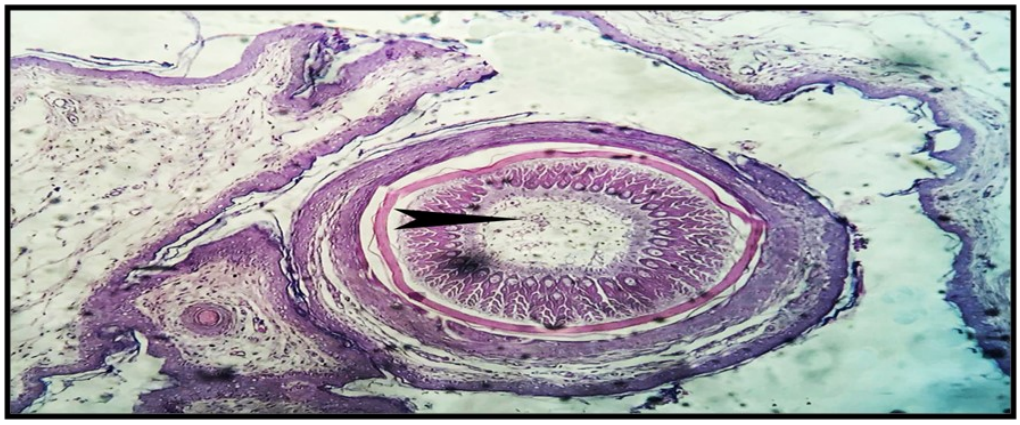

Fig. (12): Photomicrograph of joint epiphysis of 14months aged Muscovy duck showing cyst formation in the epiphysis center (H&E x100)

Table (5): Lesion score of different articular parts in ducks suffering arthritis

Affected part	Lesion	Type of infection	Lesion Severity	Lesion %
Skin	Dermal hyperkeratosis	All mixed infection included *Staph* spp.	+++	20%
	Subdermal edema	All mixed infection included *Staph* spp., *Salmonella, Klebsiella* spp. and *M.synoviae*	++	39%
	Leucocytic cells Infiltration	All single and mixed bacterial infection +*M. synoviae*	+++	70%
Tendons and Synovium	Tendonitis	All mixed infection included *Staph* spp. and *M. synoviae*	++	32%
	Fibrosis	All mixed infection included *Staph* spp. and *M. synoviae*	++	32%
Cartilage	Erosions and ulcers	All mixed infection included *Staph* spp., *Salmonella* and *M.synoviae*	+++	39%
	Ulcers	All mixed infection included *Staph* spp. *Salmonella, E coli.* and *M. synoviae*	+++	54%
	Necrosis of chondrocytes	All mixed infection included *Staph* spp. , *E coli and M. synoviae*	+++	42%
Bone	Decalcification	All mixed infection included *Staph* spp. and *M.synoviae*	++	32%
	Epiphyseal cyst formation	All mixed infection included *Staph* spp. , *Klebsiella* spp. and *M.synoviae*	++	36%

Conclusion

Great attention should be considered on studying the infectious agents underlying arthritis problem in ducks farms. *M. synoviae, S. aureus, CNS, Salmonella* spp., *E. coli* and *Klebsiella* spp. were the main causes of arthritis in ducks farms of different ages of poor hygienic conditions and low biosafety measures causing severe economic losses due to low feed conversion rate and thus condemnation.

Recommendations

Field recommendations should be put in consideration to protect ducks from arthritis which affect the feed consumption and thus the feed conversion rate causing economic losses. Here are some recommendations.

Application of strict hygienic and biosafety measures in duck farms periodically to get rid of pathogenic agents.

Clinical investigation of birds to detect early infection.

Avoid predisposing factors which initiate, facilitate and accelerate arthritis and thus lame-

ness; skin injuries, nutritional, metabolic, immunological disorders, some viral diseases,. ….etc.
The use of antibiotics of choice in treatment of different bacterial agents according to the laboratory recommendations to avoid the miss use of antibiotics and thus the organism resistance reflecting on human's health.
Vaccination programs should be strictly applied

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