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EPIDEMIOLOGICAL AND MOLECULAR STUDIES ON *RIEMERELLA ANATIPESTIFER* INFECTION IN DUCKS

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ABSTRACT

Infectious serositis is a considerable economic problem in duck industry caused by *Riemerella anatipestifer*. The current study was conducted to investigate the circulating *R. anatipestifer* in ducks in Assiut Province and assessing their antimicrobial susceptibility. One-hundred and twenty diseased or freshly dead ducks aging 1-18 weeks were examined. Naturally infected birds showed respiratory, nervous, and locomotor disturbances, and low body weight. *R. anatipestifer* was detected in 16.6% (20) of birds. Among the bacteriologically positive 20 birds, only 10 could be identified by PCR as *R. anatipestifer* with a prevalence rate of 8.33%. The sensitivity biogram revealed that all the obtained isolates were sensitive to amoxicillin, doxycycline, and flumequine while resistance to streptomycin, chloramphenicol, ampicillin, erythromycin, spectinomycin, and cephradine was observed. On the basis of MIC, all isolates had 90- 100% sensitivity to doxycycline and amoxicillin, respectively. Experimentally, the isolated *R.anatipestifer* strains showed pathogenicity to 14-days-old ducklings.

Keywords: Ducks, *Riemerella anatipestifer*, PCR, MIC, pathogenicity.

INTRODUCTION

Among the global leading problems confronting duck industry is *Riemerella anatipestifer* infection that implicates in acute and chronic conditions and can develop into epizootic infectious polyserositis in domestic ducks, mainly young, with a mortality of up to 90% (Sandhu, 2008; Wang *et al.*, 2014; Majhi *et al.*, 2020). The *R. anatipestifer* is a short to filamentous rod-shaped, Gram-negative, singly or in pairs, non-motile, non-spore-forming bacterium that is capsulated with Indian ink (Hess *et al.*, 2013; Shancy *et al.*, 2018). Clinically, the infected birds show lethargy, nasal discharge, swollen sinuses, dyspnea, diarrhea and neurologic disturbances (Sandhu, 2003; Wu *et al.*, 2020). Ducklings under 5-weeks old, die within 1 to 2 days after appearance of clinical signs, but the older may survive longer (Deif *et al.*, 2015; Shancy *et al.*, 2018). Fibrinous pericarditis, perihepatitis air-sacculitis, and meningitis with severely congested liver and spleen are the main gross lesions (Sandhu, 2008; Chikuba *et al.*, 2016; Shancy *et al.*, 2018). Conventional microbiological examination is a helping tool in *R. anatipestifer* detection but being time consuming and laborious, recent *R. anatipestifer* specific PCR method is developed revealing great success in fast,

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accurate and reliable identification of the bacteria (Wang *et al.,* 2012; Soman *et al*., 2014). Antimicrobial agents and improved biosecurity are currently applied to prevent and control *R. anatipestifer* infection in waterfowl farming; however, the increasing resistance to common antibiotics in *R. anatipestifer* seriously challenges the treatment (Chen *et al.,* 2012). Tracing the literature back, only few attempts in Assiut were done by Shahata and Sokkar, (1977); Ibrahim and Shahata, (1991); and Ibrahim and Abd Al-Azeem, (2005) to study this problem in ducks. So this study aimed at investigating incidence of *R. anatipestifer* infection in diseased ducks, and determining the antibacterial susceptibility pattern and pathogenicity of the prevalent *R. anatipestifer* isolates in Assiut Province.

MATERIALS AND METHODS

Sampling:

Altogether, 120 specimens (60 livers, 20 lungs and 40 naso-tracheal swabs) from diseased or freshly dead ducks aging 1-18-weeks old were collected under complete aseptic condition, over the period from January 2020 to September 2020, from the different diagnostic laboratories in Assiut, Egypt, and transported to the laboratory of Faculty of Veterinary Medicine-Assiut University.

Bacteriological and biochemical examination:

A methylene blue stained film was examined from each specimen. Swabs from each organ were inoculated separately into trypticase soy broth and incubated at 37ºC for 24 hours. Then, a loopful from the incubated broth was streaked onto 10%-sheep blood agar (BA) and incubated at 37ºC under anaerobic conditions for 24 hours. The suspected colonies were sub-cultured into MacConkey agar at 37 ºC for 24 hrs. and were stained with gram' stain to be examined microscopically. The purified suspected *R. anatipestifer* colonies were biochemically identified (Indole production, Urease, Catalase, Oxidase, and Litmus milk tests). Also, sugars (sucrose, glucose, lactose, fructose, maltose, dulcitol, salicin, D-mannitol, galactose) fermentation were tested according to Quinn *et al.* (2002).

Molecular identification by using Polymerase chain reaction (PCR):

Purified *R. anatipestifer* genomic DNA was obtained by boiling according to Soman *et al.* (2014). Briefly, pure colony was suspended in 5 ml of phosphate buffered saline (PBS) and centrifuged at 3000×g for 10 min at 4°C (repeated thrice until obtaining pellet). The pellet was washed twice in PBS, re-suspended in 100 µl of nuclease free water, boiled for 10 min, chilled in ice for 30 min. and centrifuged at 3000×g for 5 min at 4°C. Finally, the supernatant was collected and used as template DNA. *R. anatipestifer* species specific primer set (Forward (F): (5´-TTACCGACTGATTGCCTTCTA-3´ and Reverse (R): (5´-AGAGGAAGACCGAGG ACATC-3´) was used for amplification.

The PCR reaction mixture (25μl total volume) contained 12.5μl Master mix (One PCR^{TM} master mix (Gene Direx) Code No. MB203-0100), 9.5μl PCR grade water, 1μl Forward primer (10pM/µL), 1μl Reverse primer (10pM/µL), 1μl Template DNA (25µl total reaction). The reaction was conducted in Veriti thermocycler (Applied biosystems, Germany) following the cycling conditions described by Shancy *et al*. (2018). Accurately, an initial hot start at 94°C for 5 minutes, followed by 35 cycles, each consisting of 95°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute; and a final extension step at 72°C for 4minutes. The amplified products (5µl) were detected on ethidium bromide-stained 1.5% agarose gel by visualizing them with UV light in comparison to molecular size of 100-1.500bp DNA ladder (RTU, Cat.No.DM001. R500, 11bands).

Determining the Antibacterial Susceptibility Pattern of *R. anatipestifer* using Standard Disk Diffusion method:

In-vitro susceptibility of *R. anatipestifer* isolates to 14 antibacterial agents (Ampicillin (10µg); Amoxycillin (10µg); Gentamicin, (10µg); Streptomycin, (10µg); Spectinomycin (100µg); Cephradine (30µg); Erythromycin (15µg); Chloramphenicol (30µg); Doxycycline (30ug) Oxytetracycline (30µg); sulfa+trimethoprim (25µg) and Flumequine (30µg)) was investigated according to Bauer *et al.* (1966) following Clinical Laboratory Standards Institute (NCCLS, 1999). CLSI, 2010; and CLSI, 2018).

Detection of minimum inhibitory concentration (MIC):

The anti-*Riemerella* effect of Spectinomycin,Streptomycin, Erythromycin, Florphenicol, Sulphaquinoxaline, Doxycycline, Cephradine, Gentamycin, Amoxicillin and Lincomycin was checked in microtiter plate 96 wells using double fold micro-dilution method against all obtained *R. anatipestifer* in a density of 10^5 CFU (CLSI, 2018). The concentration of each antimicrobial was 10 µg/mL, 2.56 µl of each antimicrobial was added in 2 wells of the first row of plate then 50 µl tryptone soya broth with bacteria was added to all wells. Another 50 µl tryptone soya broth with bacteria was added to first row of plate (wells of antimicrobials) then two-fold serial dilution technique was made and discard the last 50 µl. The bacterial inoculum broth was taken as a positive control and another broth without bacterial inoculum was considered as a negative control. The microtiter plates were incubated at 37ºC for 24 hours and examined for the lowest concentration showing no detectable growth (MIC).

Pathogenicity testing:

A total number of 40 one-day-old molar ducks were purchased from (El-Shams Company, Assiut) and reared in clean well ventilated pens. Birds were divided in to two groups (20 per each) and provided with antibiotic-free commercial ration and water ad-libitum. Daily till 14 days old, tracheal swabs were obtained from each group and inoculated in trypticase soy broth for 24 hr and plated on trypticase soy agar to exclude previous Riemerella infection. At day 15, birds in the first group were inoculated intramuscularly with 0.5ml broth culture containing 10^6CFU/ml of R.anatipestifer isolate .Birds in the second group were treated with sterile broth and kept as negative control.

RESULTS

Out of the 120 examined ducks, 20 were *R. anatipestifer* infected with a prevalence rate of 16.6%. The most common encountered signs observed in the examined birds were sinusitis, decrease body weight, locomotor disturbances, nervous signs and arthritis. Pericarditis, peri-hepatitis, and airsacculitis were the main postmortem lesions (fig. 1A, B).

Isolation, Bacteriological and biochemical identification:

Methylene blue stain for tissue smears and blood films of the suspected samples showed typical bipolar cocco-bacillary organisms (fig. 2A, 2B). The produced colonies showed morphological characteristics typical to *R. anatipestifer* on the used culture media (smooth, convex, transparent, glistening, dew drop like, mucoid on the trypticase soy agar and non-hemolytic in blood agar) (fig. 3A, 3B). No bacterial growth was detected on MacConkey agar.

Gram's stained films showed gram-negative coccobacilli that were bipolar in recent cultures. The isolated suspected bacteria showed no evidence of motility on the semisolid agar media. Isolates were urease, catalase and oxidase positive, slow alkaline change of litmus milk. Indole production was negative and could not ferment sugars (glucose, fructose, maltose, sucrose, lactose, Salicin, Dulcitol, and Galactose).

Molecular characteristics of the obtained isolates:

Out of the 20 biochemically positive *R. anatipestifer* isolates, 10 isolates produced the typical band of the *R. anatipestifer* specific gene (546 bp) during the molecular examination with a prevalence rate of 8.33% (fig. 4)

Antimicrobial Susceptibility of the isolated *R. anatipestifer*:

The isolated *R. anatipestifer* showed *in-vitro* sensitivity mostly against amoxicillin, doxycycline, and Flumequine and all isolates were absolutely resistant to streptomycin, chloramphenicol, ampicillin, erythromycin, Spectinomycin, and Cephradine (Table 1, Fig. 5).

Minimum inhibitory concentration (MIC):

On the basis of MIC test for 10 different antimicrobials it was found that all isolates were resistant to 4 antimicrobials (100%) (Lincomycin, erythromycin, sulphaquinoxaline, Spectinomycin. 9 isolates were resistant to 3antimicrobials (90%) (Streptomycin, Cephradine, florphenicol). 9 isolates were sensitive to amoxicillin (100%). 9 isolates were sensitive to doxycycline (90%). 3 isolates were sensitive to gentamicin (30%). As shown in table (2), table (3) and fig. (6).

Pathogenicity test:

The ducks aged 14 days were inoculated intramuscularly with 0.5ml of broth culture containing 10^6CFU/ml of *R.anatipestifer isolate NO (1)*. Clinical picture observed after inoculation was nasal and ocular discharge, mild sinusitis, and congestion of beak, depression, ataxia, ruffled feathers, nervous signs, arthritis and greenish white diarrhea. Two birds died per acutely after 24 hours post inoculation by (I/M route) with septicemic picture (congested lung and enlarged congested liver and spleen). Post 7 days infection, necropsy findings observed as perihepatitis, air sacculitis and pericarditis as shown (fig. 7A&B, 8A&B, 9A&B, 10, 11A&B&C). Control group showed No (sings, lesions and deaths). Re isolation of the inoculated organism from experimentally infected ducks was successful.

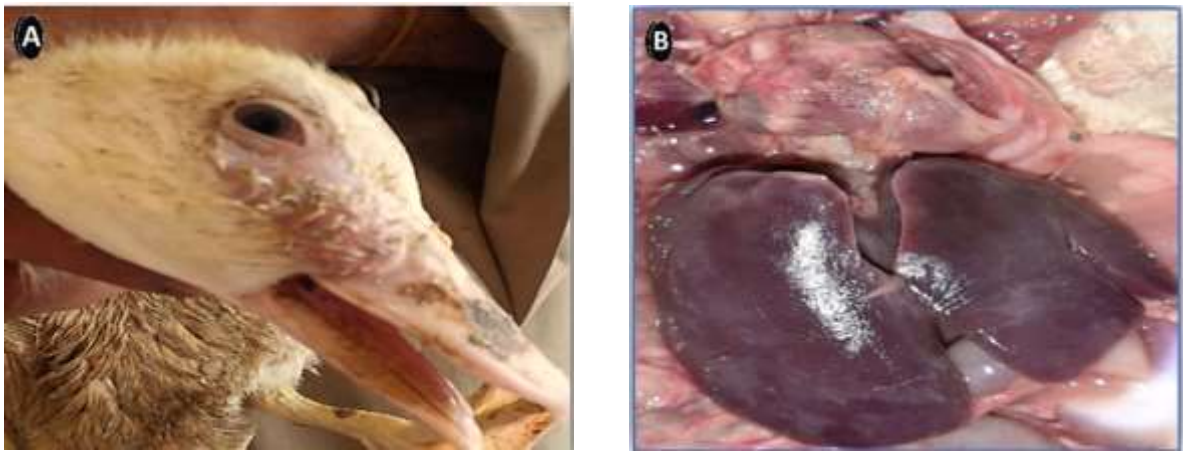

Fig. 1: (A) Sinusitis in naturally *R. anatipestifer* infected ducks. (B) pericarditis, perihepatitis, air sacculitis due to natural infection.

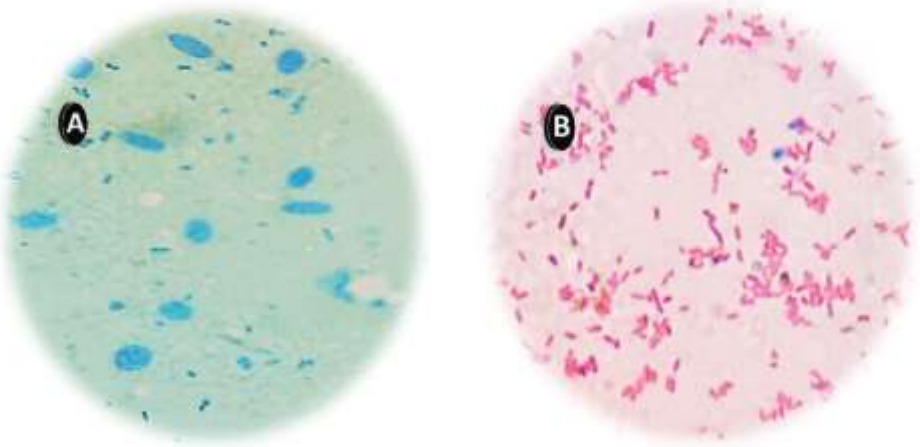

Fig. 2: (A) Methylene blue stained blood smear showing bipolar coccobacilli from *R. anatipestifer* infected duckling. (B) Gram's stained film from a recent culture showing gram negative bipolar cocco-bacilli.

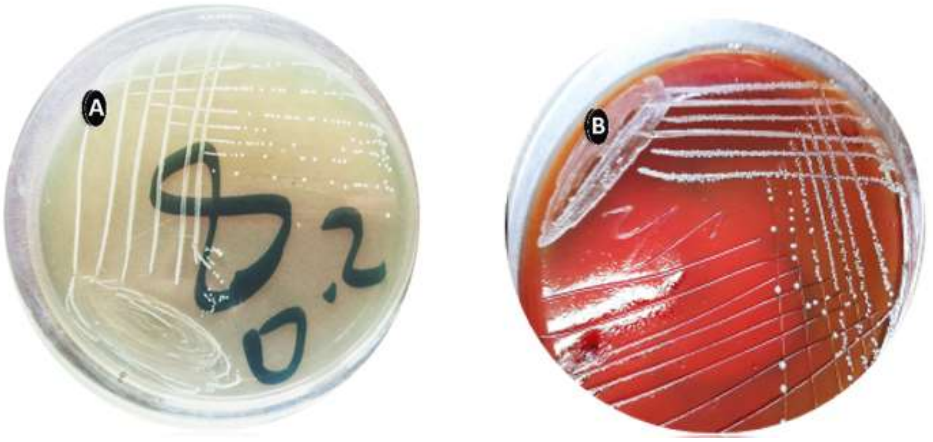

Fig. 3: (A) Dew drop like, mucoid colonies on trypticase soy agar. (B) Dew drop like non-hemolytic colonies on blood agar

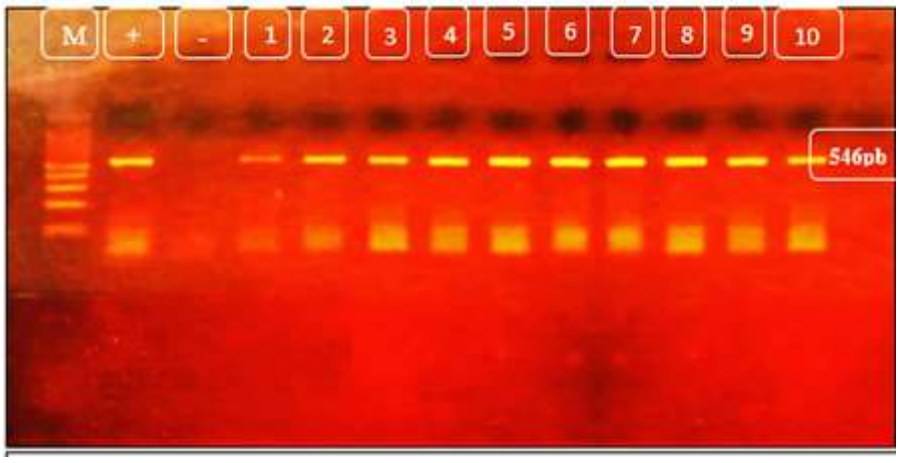

Fig. 4: Agarose gel electrophoresis 1.5% stained with ethidium bromide showing PCR products of *R. anatipestifer* specific gene illuminate (546pb) detected in biochemically positive colonies. Lane M: 100 bp ladder as molecular size DNA marker. Lane (+): control positive (pure *R. anatipestifer* strain). Lane (-): control negative. Lanes 1 to 10: Positive samples.

Table 1: Antimicrobial sensitivity profile of *R. anatipestifer* isolates.

Antibacterial agent	Resistant		Intermediate		Sensitive	
	No	%	No	%	No	%
Amoxicillin	1	10	3	30	6	60
Ampicillin	10	100	0	0	0	0
Doxycycline	2	20	3	30	5	50
Oxytetracycline	9	90	1	10	0	0
Flumequine	6	60	0	0	4	40
Sulfa+trimethoprim	9	90	0	0	1	10
Gentamicin	8	80	0	0	2	20
Streptomycin	10	100	0	0	0	0
Spectinomycin	10	100	0	0	0	0
Erythromycin	1o	100	0	0	0	0
Cephradine	10	100	0	0	0	0
Chloramphenicol	10	100	0	0	0	0

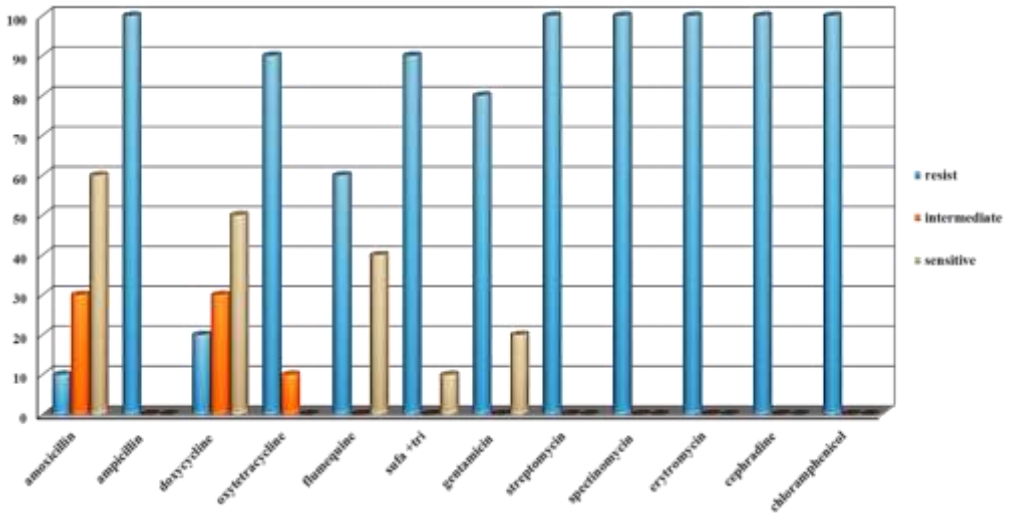

Fig. (5): Antimicrobial sensitivity profile *R. anatipestifer* isolates.

Table 2: MIC of 10 R. *anatipestifer* isolates.

Antimicrobial agents	Resist		Intermediate		sensitive	
	No.	%	No.	%	No.	%
Amoxicillin	0	0	0	0	10	100
Doxycycline	1	10	0	0	9	90
Gentamicin	7	70	0	0	3	30
florphenicol	9	90	0	0	1	10
Streptomycin	9	90	0	0	1	10
Cephradine	9	90	0	0	1	10
Spectinomycin	10	100	0	0	0	0
Lincomycin	10	100	0	0	0	0
Erythromycin	10	100	0	0	0	0
sulphaquinoxaline	10	100	0	0	0	0

Table 3: MIC breakpoint of *R. anatipestifer* isolates.

Antimicrobial agents	<2	4	8	16	32	64	128	>256	Resistance breakpoint	Resistance %
Amoxicillin	10	-	-	-	-	-	-	-	>64	0%
Doxycycline	-	-	3	6	1	-	-	-	>32	10%
Gentamicin	2	-	1	-	2	1	2	2	>16	70%
Florphenicol	-	1	-	-	-	5	4	-	>8	90%
Streptomycin	-	1	-	-	-	-	4	5	>4	90%
Cephradine	-	1	-	-	1	3	5	-	>8	90%
Erythromycin	-	-	-	-	-	-	5	5	>8	100%
Lincomycin	-	-	-	-	-	-	-	10	>16	100%
Spectinomycin	-	-	-	-	-	-	5	5	>4	100%
Sulphaquinoxaline	-	-	-	-	-	-	-	10	>256	100%

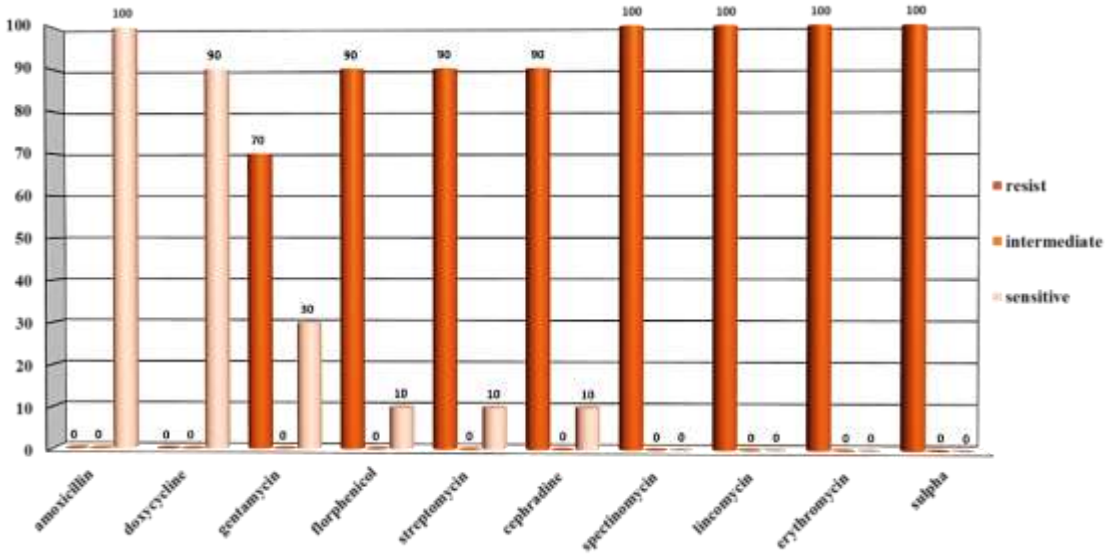

Fig. (6): MIC of 10 *R. anatipestifer* isolates.

Fig. (7): Experimentally infected ducks show diarrhea, incoordination, ruffled feathers, and anorexia.

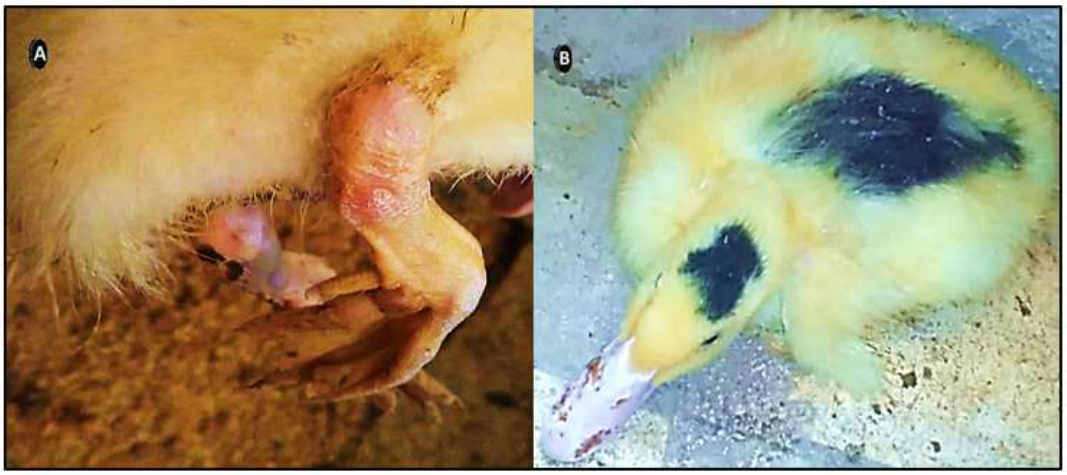

Fig. (8): Experimentally infected ducks show (A) arthritis, and (B) nervous signs

Fig. (9): (A) Experimentally infected ducks show ocular and nasal discharge and congested beak. (B): duck on the right was control and the other on the left was infected duck was infected and show loss of weight

Fig. (10): congested septicemic liver of experimentally infected duck 24hours post-inoculation.

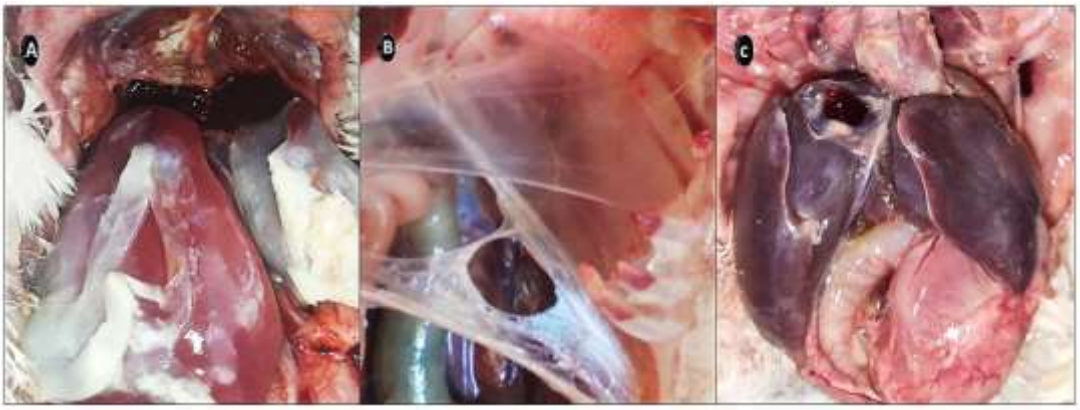

Fig. (11): Illustrates (A) perihepatitis, (B) air sacculitis and (C) pericarditis after 7-days post-inoculation of the isolated organism.

DISCUSSION

R. anatipestifer is one of the problems retarding duck industry worldwide causing infectious polyserositis of ducklings, with a mortality up to 90 % (Sandhu, 2008; Wang *et al.,* 2014; Majhi *et al.,* 2020). Our study was designed to determine the prevalence of *R. anatipestifer* infection in ducks and evaluate the antibacterial susceptibility and pathogenicity of the obtained *R. anatipestifer* isolates. In the current work, 20 suspected isolates of the organism were recovered from different duck farms in Assiut Province.

The most common encountered signs observed in the examined birds were sinusitis, decrease body weight, locomotor disturbances, arthritis and nervous signs. These findings were recorded by several authors as Leibovitz, (1972); Leavitt and Ayroud, (1997); Sandhu, (2003); Aparna and Renjith, (2012); Hess *et al*. (2013) and Wu *et al.* (2020). Postmortem lesions included lungs congestion, enlarged pinkish liver and enlarged purple spleen, enteritis, congested beak, pericarditis, perihepatitis, airsacculitis, meningitis, osteomyelitis, caseous salpingitis, chronic arthritis and cellulitis. These lesions aligned with those recorded by Aparna and Renjith, (2012).

The produced colonies showed morphological characteristics typical to *R. anatipestifer* on the used culture media. The colonies were smooth, convex, transparent, glistening, dew drop like, mucoid on the trypticase soy agar and non-hemolytic in blood agar, no bacterial growth was detected on macConkey agar. These findings matched with Leavitt and Ayroud, (1997); Shancy *et al.* (2018) and Majhi *et al.* (2020). On the other hand some isolates were haemolytic, particularly when plates incubated longer than 48 hrs (Brogden, 1989; Shancy *et al.,* 2018). Among 123 field strains of *R. anatipestifer*, Hinz *et al*. (1998) recorded that 25 strains showed β-haemolysis on blood agar after 24h to 48h incubation.

Concerning cellular morphology of our *R. anatipestifer* isolates, they appear as bipolar cocco-bacillary organisms by methylene blue stain and gram-negative coccobacilli that were bipolar in recent cultures by gram's stain. These results are in agreement with these described by Pillai *et al.* (1993); Hess *et al*. (2013); Pala *et al.* (2013) and Shancy *et al.* (2018).

In the present study, biochemical tests revealed that *R. anatipestifer* isolates were slow alkaline change of litmus milk; indole production was negative while oxidase, catalase and urease were positive. These findings agree with (Brogden *et al*., 1982; Soman *et al.,* 2014; Deif *et al*., 2015 and Surya *et al.,* 2016). The results of sugar fermentation of our isolate revealed that most isolates could not ferment sugars including (glucose, fructose, maltose,

sucrose, lactose, salicin, dulcitol, and galactose). These findings were also reported by (Brogden *et al.,* 1982 and Bernardet *et al*., 2002). On the other hand, Priya *et al.* (2008) and Deif *et al*. (2015) reported that this bacteria ferments mostly lactose, maltose, dextrose and sucrose.

Bacteriological examination revealed a recovery of 20 *R. anatipestifer* isolates with a prevalence rate 16, 6%. These results agreed with previous studies reported by Ibrahim and Abd Al-Azeem, (2005). The prevalence rate of *R. anatipestifer* infection ranged from 11% - 84.4%% in ducks worldwide as observed by Huang, (2008); Priya *et al*. (2008); Wang *et al.* (2012); Chen *et al.* (2015) and Majhi *et al.* (2020). Seventy-six *R. anatipestifer* isolates were detected, and the prevalence in the ducks and geese were 12.3% (46/375) and 8.0% (30/375), respectively in central Taiwan recorded by Chang *et al*. (2019). In contrast, Deif *et al*. (2015) reported that recovery of 20 R. anatipestifer isolates with a prevalence rate 16.7%. Out of 69 samples collected from diseased ducks (more than 7 weeks old) revealed isolation of 14 R. anatipestifer isolates (11.7%). While out of 51 samples collected from diseased ducklings (1-7 weeks old), revealed isolation of 6 isolates (5%). Comparing the results obtained from ducks and ducklings showed a higher prevalence rate of ducks (11.7%) than ducklings (5%).

According to molecular characterization 10 out of 20 *R. anatipestifer* isolates with prevalence rate (8, 33%) were positive in PCR assay which is considered to be a useful laboratory tool for the definitive identification of suspected R. anatipestifer isolates due to absence of selective and/or indicative media for isolation (Rubbenstroth *et al*, 2009), it was some time difficult to isolate the organism from clinical samples due to overgrowth of other organism. These was described by Higgins *et al*. (2000) and Cultural and biochemical characteristics based identification of *R. anatipestifer* is time consuming, laborious, and require several days to complete (Soman *et al*., 2014). Characterization of *R. anatipestifer* by traditional methods is often not sufficient because of phenotypic diversity (Deif *et al*., 2015). Our results are in disagreement with those described by Wang *et al.* (2012) who revealed that Using *gyrB*-PCR to livers of diseased ducks, 46% riemerellosis incidence rate was recorded in China.

In our recent study, the results of antimicrobial susceptibility test of the isolates showed that the most effective antibiotics were amoxicillin, doxycycline, Flumequine. While all isolates showed absolutely resistant to streptomycin, chloramphenicol, ampicillin, erythromycin, Spectinomycin and Cephradine. Sandhu, (2001) stated that Sulfadimethoxine-trimethoprim was effective in reducing mortality. According to Chang *et al.* (2003); Zhong *et al.* (2009) and Deif *et al.* (2015), all isolates were of high sensitivity to Ciprofloxacin, Norfloxacin, Gentamycin, Chloramphenicol, Polymyxin-B and with moderate sensitivity to Doxycycline, and resistance to penicillin G, Metronidazole, Sulfadiazine, Methicillin, Ampicillin, Cefuroxime, Erythromycin. On the basis of MIC test for 10 different antimicrobials it was found that all isolates were resistant to 4antimicrobials (100%) (Lincomycin, erythromycin, sulphaquinxaline, Spectinomycin. 9 isolates were resistant to 3antimicrobials (90%) (Streptomycin, Cephradine, florphenicol). All isolates were sensitive to amoxicillin (100%). Nine isolates were sensitive to doxycycline (90%). 3 isolates were sensitive to gentamicin (30%). Ibrahim and Hussein, (2000) and Ibrahim Abd Al-Azeem, (2005) studied susceptibility of *R. anatipestifer* to different antimicrobials using MIC, they recorded complete susceptibility to penicillin, amoxicillin, enrofloxacin, lincospectin, Oxytetracycline and cephalosporin. Complete resistance to aminoglycosides (streptomycin, gentamycin) and sulfadimethoxin was demonstrated.

The recorded gross lesions in birds that exposed to the experimental infection with *R. anatipestifer* were pericarditis, perihepatitis, air sacculitis and septicemic lesions especially in liver, spleen and myocardium. Our observation on gross lesions are similar to findings of Tripathy *et al*. (1980) and Ibrahim and Shahata, (1991) who reported that pericarditis and variable degree of air sacculitis were common lesions, Bayoumi, (1988) who recorded hemorrhage and septicemic picture and Hatfield and Morris, (1988) who observed clinical signs of diarrhea and incoordination in ducklings , severe pericarditis , thickening of air sacs and fibrinous pericarditis in intramuscularly inoculated group of ducklings, Per-acute death with septicemic picture had been seen by Ibrahim and Abd Al-Azeem, (2005). The current results are in disagreement with those described by Asplin, (1956) who said that no clinical signs were observed in any experimental group of duckling.

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دراسات وبائيه وجزيئيه على الاصابه بميكروب رايميريللا اناتيبيستيفيرفى البط

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اجريت هذه الدراسه بهدف عزل وتشخيص ميكروب الريميريلا اناتيبيستيفير في البط فى محافظه اسيوط. تم جمع ١٢٠عينه (٦٠ عينه من الكبد ،٢٠ رئه ،٤٠ قصبه هوائيه ، مسحه من الانف) من بط مصاب او نافق حديثا يترواح عمره من اسبوع ال ١٨ اسبوع في الفتره من شهر يناير الي سبتمبر ٢٠٢٠. تم مشاهده الاعراض المرضيه علي البط علي شكل افرازات من الانف والعين , ضعف النمو,خمول,اعراض عصبيه مع عدم القدره علي المشي .وتم تسجيل الصفه التشريحيه. اسفرت نتائج االاختبارات البكترولوجيه المستخدمه لعزل الميكروب والتعرف عليه (مورفولوجيا المستعمرات البكتيريه والخلايا والاختبارات البيوكيمائيه والزرع علي الماكونكى) عن عزل عشرون (٢٠) عتره محتمله للميكروب قيد الدراسه وذلك بنسبه ١٦,٦% علما بان الميكروب لم ينمو علي بيئه الماكونكي اجار. للتعرف الجزئيى تم اجراء تقنيه تفاعل البلمره المتسلسل علي العترات المعزوله (٢٠) والذى اظهرعشر عينات ايجابيه لبكتريا الرايميريلا اناتيبيستيفر بنسبه عزل كليه ٨,٣٣%.تم اجراء اختبار الحساسيه باستخدام عدد ١٢ من المضادات الحيويه المختلفه لدراسه مدي تاثيرها علي العترات المعزوله والتى بينت ان معظم المعزولات حساسه لكل من الاموكسيللين ,الدوكسيسيكلين وعدم حساسيتها لكل من ستريبتومايسين , الكلورمفينيكول ,الامبيسيلين,الايثرومايسين ,السبكتينومايسين والسيفرادين. عند دراسه مدى تاثير اقل جرعه مثبطه من المضادات الميكروبيه علي ميكروب الرايميريلا اناتيبيستيفر وجد ان جميع المعزولات مقاومه بنسبه ١٠٠% لكل من الينكومايسين ,الاريثرومايسين ,والسلفاكينوكسالين والسبكتينومايسين. ٩٠% لكل من الستريبتومايسين , السيفرادين و الفلورفينيكول وجد ان جميع المعزولات حساسه بنسبه ١٠٠% للاموكسيلين و٩٠% للدوكسيسيكلين وبنسبه ٣٠% للجنتاميسين. تم اجراء اختبار العدوى الاصطناعيه بالعتره رقم واحد للميكروب المعزول في البط عمر ١٤ يوم حقنا في العضل وتم تسجيل الاعراض الاكلينيكيه والصفه التشريحيه ونوقشت النتايج .