Study on remirella anatipestifer in ducks in Sharkia governorate

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Abstract
A total of 170 duckling were collected from different location in Sharkia Governorate either in moribund state or freshly dead. The examined samples, yield 5 (0.3%) positive *Remirella anatipestifer* isolates from different organs. The therapeutic antibiogram of *Remirella anatipestifer* isolates showed that the isolates highly sensitive to folorphenicol, sulphaqunixaline and streptomycin and resistance to colestin, ampicillin and Flemequine. One hundred duckling were experimentally infected with bacterial isolates and divided to four equal group, 1, 2, 3 and 4. Group (1) was served as control (non infected and non treated). Groups 2, 3 and 4 infected with *Remirella anatipestifer* isolates. Group 3 treated with folorphenicol meanwhile group 4 vaccinated with *Remirella anatipestifer* inactivated vaccine. The pathological findings showed petechial and echymotic heamrrhage on the abdominal fat and pericardial sac. coagulative necrosis and hydropic degenerate were detected in the liver. The study concluded that florophenicol was effective for controlling *Remirella anatipestifer* but the vaccination was more effective in controlling *Remirella anatipestifer* infection.

Key words:
Remirella, anatipestifer, Turkeys, foloramphincol.

Introduction

Riemerella anatipestifer organisms are Gram negative, non motile, non sporulating rods. The organism occurs singly, in pairs or in short chains. The cell varies from 0.2 to 0.4 micrometer in length. Many cells stains bipolar with Wrights stain and capsule can be demonstrated in Indian ink preparation **(Sandhu and Dean, 1979)**.

The organism grows well on chocolate agar, blood agar or trypticase soya agar. Growth is more abundant with increased carbon dioxide. Maximum growth usually occurs in 48-72 hours when incubated at 37ºC in candle jar. Colonies on blood agar, when grown 24 hours at 37^{o}C in candle jar are convex, entire, transparent, glistening **(Hirsh *et al.,* 2004)**. Carbohydrate are not fermented. Gelatin is usually liquefied, indole and hydrogen sulphide are not produced, nitrate is not reduced to nitrite and there no growth on MacConkey agar.

Ducklings 1-8 week of age are highly susceptible. Ducklings less than 5 weeks of age usually die 1-2 days after appearance of clinical signs. The disease is rare in breeding ducks **(Aprana and Renjith 2012)**. The disease started in 7-10 - week-old ducks with 10% mortality and later spread to younger ducklings of about 3 weeks of age. Adverse environmental conditions often predispose the birds to outbreaks of *P. anatipestifer* infection **(Singh and The 1984; Sokkar *et al.,* 1986, and Aprana and Renjith 2012)**.

This work was planned to study clinical signs and pathological findings in flocks of ducks natural or experimentally infected with *R. anatipestifer*, moreover isolation and attempts of treatment conducted.

Material and Methods

Field study.

One hundred and seventy ducks either freshly dead or moribund with average 7-80 days–old

of different breeds were collected from different localities at Sharkia Governorate and subjected to either clinical and or postmortem examination. Specimens from heart, liver lung and kidney were aseptically collected and subjected for bacterial isolation and identification.

Isolate preparation.

Cultivation and isolation of *Riemerella anatipestifer* was carried out after **(Lee *et al.,* 1991 and Rimler *et al.,* 1997)**.

Identification of *Riemerella antipestifer* by morphological, biochemical and serological according to **(Cruickshank *et al.,* 1975 and Quinn *et al.,* 2002)**.

Antibiogram.

A disc diffusion technique adapted as it was recommended by using pure culture from the testes isolates according **(Kolmar *et al.,* 1951)**.

Experimental study.

One hundred and five one day Pakeni ducklings were obtained form Al Adway Hhatchery were feed on balanced ration free from medication. The Pekin duckling were proved to be free from *Riemerella anatipestifer*. One hundred duckling were divided into four equal groups (1, 2, 3 and 4) of 25 ducklings each were reared in separate unite and fed on ration without any medication. Group (1) were kept as negative control (non-infected and non treated). Meanwhile group 4 were vaccinated with dead *Riemerella anatipestifer* 4 vaccine one day old S/C at dose 0.5 ml/bird.

At 14 day-old groups (2, 3 and 4) were injected S/C route with a dose 1 10^8 ×cfu/ml of nalidexic acid resistant *Riemerella anatipestifer* isolate.

Groups 2 ducklings were remained as positive control, Group 3 were treated with a dose 20mg/L florophencol in drinking water for 5 successive days.

Two week post treatment all rest duckling were subjected to determination of body weight gain; 5 duckling were sacrificed from each group for P.M, bacteriological isolation and histopathological examination.

Results

Bacteriological isolation.

The result of bacteriological isolation from examined freshly dead duckling was manifested in table (1). *Riemerella anatipestifer* were isolated. *Riemerella anatipestifer* detected from liver by percentage 100%, lung 40% and heart 20%.

Table (1). Incidence of *Riemerella antipestifer* among examined duckling.

No. of examined ducklings	R. antpest		Liver		Lung		Heart		Kidney	
	No	%	No	%	No	%	No	%	No	%
170	5	0.3%	5	100	2	40%	1	20%	0	0

Identification of *Rimerella* isolate from examined duckling.

Colonial appearance. on blood agar appear small, convex and transparent. Gram -ve and microorganism can grow on MacConkey's agar and had bipolar character in recent colonies.

Biochemical character. The isolate, were indole negative and urease positive, not fermented any type of sugars and H_2S production negative.

In vitro sensitivity of *Riemerella anatipestifer* isolates to antibacterial agent were conducted. isolates were sensitive to ceprofloxacine, florphenicol, sulfaquinoxalin, lincomycin, spectinomycin (Table 2).

Table (2). Result of *in vitro* sensitivity isolate of *Riemerella antipestifer*

Antimicrobial agent	Disk polancy	Standard sensitivity zones	
Florphencol	10 ug	> 18 > 23	+++
Sulfaquanoxaline	30 ug	> 18 > 23	+++
Streptomycin	10 ug	> 18 > 23	++
Enrofloxancine	10 ug	> 18 > 23	++
Gentamycin	10 ug	> 18 > 23	++
Lincomycin	10 ug	> 18 > 23	+
Spectinomycin	10 ug	> 18 > 23	+
Colestine	30 ug	> 18 > 23	R
Penicillin	30 ug	> 18 > 23	R
Ampicillin	30 ug	> 18 > 23	R
Flemequine	30 ug	> 18 > 23	R

+ = low sensitivity ++ = moderate sensitivity +++ = sensitive R = resistance

Clinical and pathological findings.

2 days post infection in group (2) clinical symptom were in the form of depression, ruffled feathers and greenish diarrhea. Difficult breathing, coughing, sneezing in 12/25 inoculated group. Mortality rate 6/25. Postmortem examination revealed congestion of internal organs. Fibrinous, pericarditis and fibrenous perihepatitis. Histopathological examination, the primary tissue showed pneumonic areas characterized by leukocytic infiltration mainly heterophils (Fig. 1); later on the suppurative and necrotic process became invaded with granulation tissue resulting in organization of exudates and emphysema (Fig. 2).

The menings of brain showed suppurative and fibrinous manifested by suppurative exudates in covering brain (Figs. 3 & 4). Focal aggulative necrosis in hepatic tissue (Figs. 5). Mild pericarditis characterized by thickened pericardium with odema and leukocyte infiltration mainly heterophils beside extravasated erythrocytes (Figs. 6).

These findings were subsided post treatment with florpheniclo and vaccinated with inactivated *R. anatipostiver* vaccine. Whose mortality rate 1/25 in group 3 with mild lesion (Table 3).

The influence of injection with *R. anatipostiver* and treatment on body weight, feed conversion and FCR. The mean body gain untreated chicks was significantly ($P<0.05$) decreased than infected and treated or vaccinated (Table 4).

Table (3). Mortality rate lesion, Reisolation of *Remirella anatipestifer* following treatment with thrphencol 20mg/k B.W. for 3 successive days.

Group	%Morbidity	Mortality	Lesion	Frequency reisolation
1	0	0	0	0
2	48%	24%	100	100
3	4%	0	0	20
4	0%	0%	0	0

Table (4). Average body weight, gain, feed consumption and feed conversion rate (FCR) for duckling vaccinated, inactivated R. ant vaccine, infected and treated with 20mg/b.wt.

Group	Initial body weight	2nd week post treatment			
		Body weight	Body weight gain	F.C	F.C R
1	340 ± 20	680 ± 25[a]	340 ± 55[a]	680 ± 55	2.5
2	345 ± 15	543 ± 48[c]	198 ± 42[c]	535 ± 62	2.7
3	350 ± 22	658 ± 32[b]	308 ± 32[b]	646 ± 62	2.1
4	344 ± 12	675 ± 24[a]	331 ± 28[a]	662	2.5

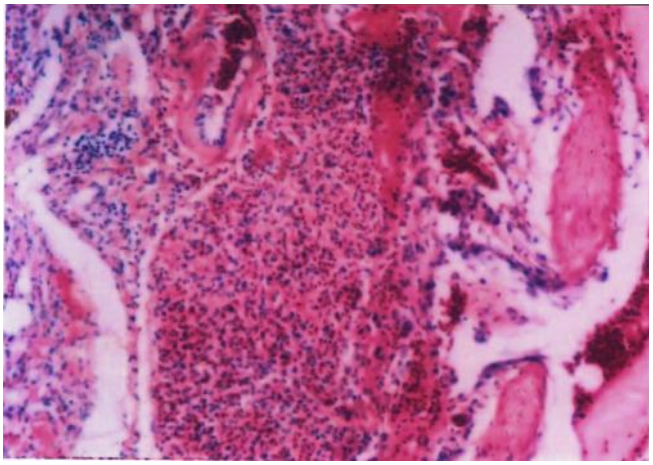

Fig. (1). Lung of duck showing pneumonia (H & E X 300).

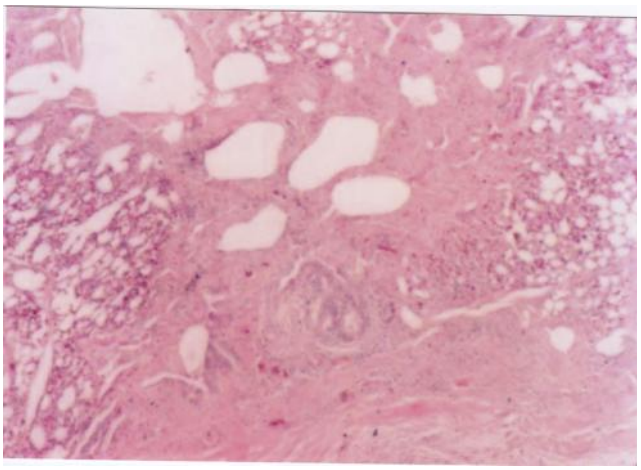

Fig. (2). Lung of duck showing organized oxudate and emphysema (H & E X 300).

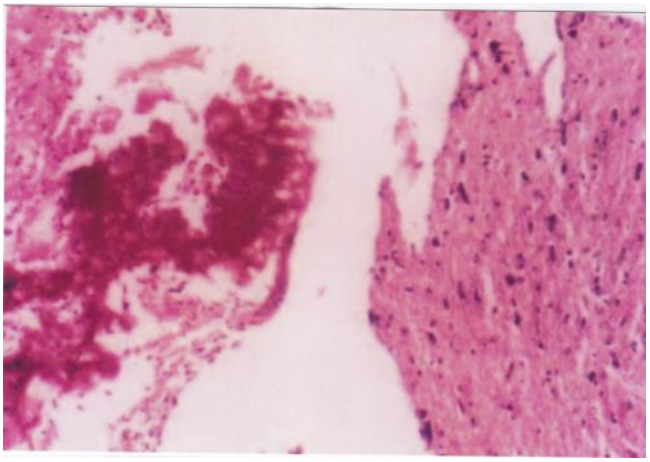

Fig. (3). Brain of duck showing organized suppurative meningitis (H & E X 300).

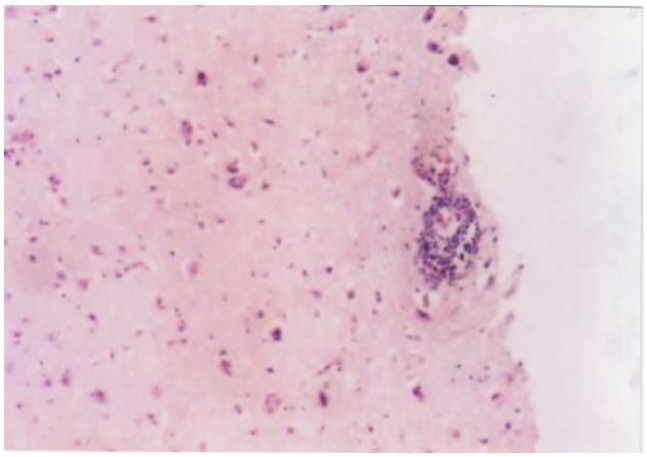

Fig. (4). Brain of duck showing submeningeal leukocytic aggregation (H & E X 300).

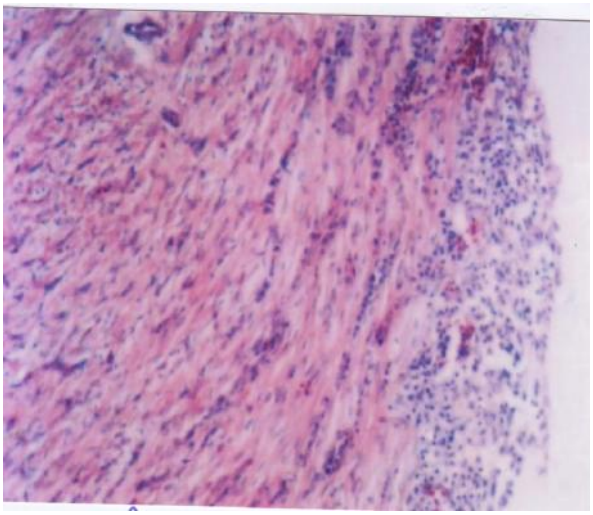

Fig. (5). Spleen of duck showing focal coagulative necrosis (H & E X 300).

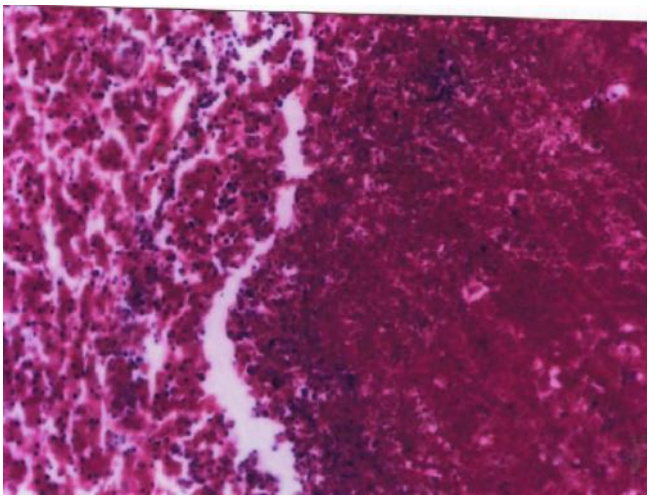

Fig. (6). Heart of duck showing pericarditis (H & E X 300).

Discussion

In our study ducks naturally infected with *Remirella anatipestifer* showed clinical signs as anorexia, ruffled feather, mucous discharge from mouth and diarrhea, these results were similar results obtained by **(Guo–Yujang, 1983)**, also our results similar to those obtained by **(Singh and The, 1984 and Brogden, 1989)** who mentioned that *Remirella anatipestifer* affect primary the respiratory tract and nervous system and result of pericarditis, perihepatitis and meningitis.

In this study, the examined sample, yielded 5 isolate of *Remirella anatipestifer* from different organs with an incidence 0.3%, these results agreed with **(Samad Poour *et al.,* 2002)**.

The antibiogram pattern of isolated pathogens from naturally infected duckling by *Remirella anatipestifer* showed that the isolate were highly sensitive to florphenicol, sulfaquanoxaline and resistance to colestin, ampicillin and flemequine. These result agreed with **(Radad and Moustafa 2005)**.

Experimentally infected with isolated *Remirella anatipestifer* were showed clinical signs in the form of sever respiratory signs. These result were agreed with result obtained from **(Sandhu and Dean, 1979 and Guo–Yujiang, 1983)**.

Histopathological changes in group (2) which detected, leukocytic infiltration mainly heterophils; later on the suppurative and necrotic process became invaded with granulation tissue resulting in organization of exudates and emphysema in internal organs were similar with that recorded by **(Guo– Yujiang, 1983 and Singh and The, 1984)**.

The treatment with florphenicol was more effective for controlling infection *Remirella anatipestifer* supporting it is still effective lead decreasing mortality from 24% to zero%. The same result was obtained by **(Radad and Mostefa, 2005)**. Meanwhile vaccinated duckling with *Remirella anatipestifer* inactivated vaccine is more effective in controlling *Remirella anatipestifer* infection.

All performance parameters were significantly lower in ducks, at the 2nd week post infection in comparison with the control group and vaccinated group. These result similar that obtained by **(Singh *et al.,* 1982)**.

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دراسات علي الريميلا أناتي بستيفير في البط بمحافظة الشرقية

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الملخص العربى

تم تجميع 170 عينة من صغار البط تتراوح أعمارهم من 7-80 يوم تمثل جميع مراكز الشرقية وهذه الطيور تعانى من ارتفاع نسبة النفوق وأعراض مرضية. وكانت الصفة التشريحية تضخم عضلة القلب وتنقرز الكبد والطحال مع التهاب رئوي ، كما تم عزل 5 عترات من الريميلا أناتي بستيفير بنسبة 0.3 %.

وبعمل اختبار حساسية للمعزولات وجد أنها حساسة للفلورفينيكول والسلفاكينوكسالين والاستربتوميسين ومقاومة للكولستين والفليموكوين والأمبسلين.

وتم إحداث العدوي الصناعية على 100 بطة عند عمر 21 يوم وكانت هناك مجموعة ضابطة ومجموعة محصنة ضد نفس المرض ومجموعة معدية وغير معالجة ومجموعة محصنة ومعالجة بالفلورفينيكول.

ومن هذه الدراسة وجد أن مقاومة هذا المرض بحقن البط الصغيرة عند عمر يوم أو العلاج عند الإصابة الطبيعية بالفلورفينيكول تقلل نسبة النافق ويحسن الوزن المكتسب ونسبة التحويل الغذائي.