

## **ORNITHOBACTERIUM RHINOTRACHEALE (ORT) INFECTION IN RABBITS**

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### **ABSTRACT**

*This work was aimed to investigate the epidemiology and possible ways of diagnosis, treatment and control of Ornithobacterium rhinotracheale (ORT) as an emerging respiratory pathogen in rabbits during the period from 2013-2015. For this purpose, bacteriological, biochemical, serological and histopathological examinations for the collected samples from suspected diseased 300 rabbits at different districts in Kafrelshiekh Governorate suffered from respiratory signs with nasal Discharge, depression with ruffled fur, decreased food consumption with poor performance and expectoration of blood stained mucus just prior to death . The results revealed isolation of 21 isolates with an incidence of 7%. The isolates were identified morphologically and biochemically by API 20 NE strip. Serological identification of the isolated strains using agar gel precipitation test revealed that all isolated ORT strains were belonging to serotype A. Antibiogramme of the isolated ORT strains revealed sensitivity to sulphamethoxazole + trimethoprine, spiramycin, neomycin, ampicillin, amoxicillin, ciprofloxacin and tetracycline but resistant to penicillin, streptomycin, clindamycin, lincomycin, gentamycin, vancomycin and*

*colistin sulphate.*

*Experimental infection of 3-month-old rabbits with isolated ORT strains and treatment trials using sulpha-trimethoprime and coconut oil were carried out. Clinical signs, post-mortem lesions and mortality with re-isolation of the infected strains were studied in details. Histopathological examination of different organs from experimentally infected rabbits revealed less severe lesions after treatment with sulpha-trimethoprime and coconut oil, in comparison with naturally infected rabbits.*

## INTRODUCTION

Commercial rabbit enterprises is an important industry for meat, fur and leather production. Respiratory infections are the most serious group of diseases affecting rabbits and accompanied by heavy economic loss due to increased mortality, increased condemnation rates, reduced fertility and medication costs. Several pathogens are indicated as possible causes of respiratory disorders either alone or in synergy with other micro-organisms.

The severity of clinical signs, duration of the disease, and mortality of *Ornithobacterium rhinotracheale* (ORT) outbreaks are extremely variable. They can be influenced by strain virulence, environmental factors, the immune status of the host, and the presence of other infectious agent (*Van Empel et al., 1999*).

ORT is a Gram-negative non-motile pleomorphic rod shaped bacterium which has been isolated from domestic birds with respiratory problems in Europe, South Africa, Egypt, Israel and USA, ORT has been

isolated from chickens (*Vanda mme et al., 199; El-Gohary,1998; and Roussan et al 2011*) and turkey (*Hafez, 1996*).

The organism has been isolated from rabbits suffered from respiratory disorder in Egypt (*Attia, 2008*), ORT has been reported to be associated with clinicopathological effects in Muscovy and Balady ducks (*El-Abasy 2008*).

ORT is difficult to be differentiated from other respiratory pathogens affecting rabbits specially *Pasteurella multocida* infections duo to colonies of ORT on culture may resemble those of *Pasteurella multocida* on routine isolation media and cause confusion in confirming.

ORT infection can be successfully treated with antibiotic, but the bacterium rapidly developed antibiotic resistance (*Devriese et al., 2001*). So the aim of this work was to investigate the incidence. isolation and identification of ORT and treatment trials of ORT experimentally infected rabbits.

## MATERIALS AND METHODS

### 1. Collected Samples:

Samples have been collected from 40 rabbit farms distributed in different localities in Kafrelshekh Governorate suffering from respiratory manifestation were examined for presence of ORT during the period from 2013 to 2015. Samples were taken under aseptic condition in ice tanks to the laboratory. The history of investigated rabbit farms, clinical signs, postmortem lesions as well as mortality rate were described in Table (1).

### 2. Bacterial Isolation:

Under aseptic condition loopfuls from (sinus, trachea, lung, and liver) of diseased rabbits were inoculated in brain heart infusion broth and incubated at 37°C for 24 hours. A loopful from broth into 10 % sheep blood agar media containing 10 µg gentamicin sulphate/ ml blood agar media to inhibit the over growth of the other bacteria (*Back et al.,1996*) and incubated at 37°C for 24-48 hours under 7.5% CO<sub>2</sub> tensions. Suspected ORT colonies, after purification were identified morphologically (shape, size, colour, appearance, odour, and elevation). Finally films were prepared from the suspected pure colonies stained with Gram's stain and examined microscopically (*Chin and Charlton, 2000*).

### **3. Biochemical identification:**

Suspected colonies of ORT were inoculated into buffer peptone water at 37° C for 48 hours under 7.5 CO<sub>2</sub> tension. Using micropipette or syringe for inoculation of this culture into different tests of API 20 NE strip, incubated at 30° C for 48 hours. The interpretation of the test was carried out according to the instruction of the kits producer (*Hafez, 1996*).

### **4. Serological identification:**

Suspected ORT strains were serologically identified by using agar gel precipitation test (AGPT) (*Van Emple et al, 1999*). Approximately 6 ml of sterile agar in 60 mm petri-dish was used to obtain 2.8 mm thickness. Patterns consisting of six or seven wells (3 mm in diameter) located around a central well at a distance of 5 mm were punched out the agar. The central well was filled with the antigen extract and the

peripheral ones with the anti-sera against ORT organism. The plates were incubated at room temperature and evaluated after 24, 48 and 72 hours. The observation for perception lines occur under ultra violet light.

### **5. Experimental infection:**

Thirty 3-month-old hybrid rabbits obtained from rabbit farm of Faculty of Agriculture Collage, Kafrelshickh University were used for pathogenicity test. Fed green clover and pellet ration, the food and water were added *ad-libtum*. Rabbits were divided into two groups each group contained 15 rabbits. Each group. divided into three subgroup of 5 rabbits each in separate box. The first group was infected by ORT serotype A by an aerosol in dose  $1 \times 10^9$  C.F.U/ml, and the second group was kept uninfected kept under observation for 4 weeks.

### **6. Antibiogramme:**

The antibiotic sensitivity test of the isolated ORT strains were investigated against 19 antimicrobial agents using disc diffusion technique. The colony of ORT was picked up and suspended in tryptose soy broth to a turbidity of 0.5 McFarland scale. One hundred microliters of the culture was spread uniformly onto the different media:

- Muller Hinton agar supplemented with 10% sheep blood.
- 10% sheep blood agar

The discs were dispensed on the culture and the plates were incubated at 37°C for 24 hours under micro aerophilic condition .The inhibition zones were measured according to National Committee for Laboratory Studies (*Back et al., 1997*).

### **7. Treatment Trials:**

Based on the result of the in vitro antibiogramme, sulphamethoxazole (SXT) and coconut oil were used for treatment trials of ORT experimentally infected rabbits.

## 8. Histopathological examination:

Specimens of sinus, larynx, trachea, lung, heart, liver and kidney were taken from freshly dead and sacrificed experimentally infected rabbits, fixed in 10% neutral buffer formalin (*Lillie,1984*), washed, dehydrated in different concentration of alcohol, cleared in xylol and embedded in paraffin. The paraffin embedded blocks were cut into 5µm section and stained with Haematoxylin and Eosin (H &E) stain according to *Bancraft and Gamble (2007)*.

## 9. Statistical analysis:

Statistical analysis of obtained data was carried out according to *Petri and Watson (1999)*.

**Table (1):** History of investigated rabbit farms in Kafrelsheikh Governorate

Farm No.	Districts	No. of Rabbits in Flock	Breed	Clinical signs	Postmortem Lesions	Mortality rate	
						No.	%
1	Kafrelsheikh	600	New Zealand	Sneezing, cough and nasal discharge	Trachitis and pneumonia	40	6
2	Kafrelsheikh	900	New Zealand	Bloody mucoid discharge	Septicemia	5	0.5
3	Kafrelsheikh	500	Dutch	Coughing and nasal discharge	Sinusitis, trachitis and consolidation of lung	5	1
4	Sakha	1000	Hybrid	Mucoid nasal discharge and conjunctivitis	Congested lung and yoghourt like exudates in the thoracic cavity	6	0.6
5	Sakha	1000	Hybrid	Dyspnea and poor growth	Trachitis and unilateral pneumonia	4	0.4
6	El-Reyad	300	New Zealand	Mucoid nasal discharge	Trachitis, sinusitis and haemorrhagic spots in lung	10	3.3
7	El-Reyad	500	Bosuchate	Mild respiratory sings and abortion	Trachitis, sinusitis and congested in uterus	8	1.6
8	El-Hamol	500	Hybrid	Poor fur and respiratory sings	Trachitis and haemorrhagic spots in lung	5	1.0
9	Kafrelsheikh	600	Hybrid	Rales, nasal discharge and poor weight	Trachitis, pneumonia and hydrothorax	4	0.6

10	Kafrelsheikh	500	New Zealand	Sneezing, cough and emaciation	Trachitis, pneumonia and hepatomegally	3	0.6
11	Araymon	800	New Zealand	Dyspnea ,cough and,nasal discharge	Trachitis, pneumonia and congestion of liver	6	0.75
12	Dosque	600	Hybrid	Rales, nasal discharge and ocular discharge	Trachitis, pneumonia and pyothorax	3	0.5
13	Dosque	500	Bosuchate	Coughing,sneezing and conjunctivitis	Trachitis, pneumonia and hydrothorax	5	1
14	Foa	1000	Hybrid	Mild respiratory signs and salivation	Slight congestion in trache and lung	20	2
15	El-Hamol	1000	New Zealand	Dyspnea, coughing and mucoid nasal discharge	Trachitis and pneumonia	5	0.5
16	Motobes	500	New Zealand	Rales, coughing, ruffled fur, ocular discharge and low body weight	Trachitis, pneumonia and enlargement of liver	10	2
17	Kafrelsheikh	900	New Zealand	Coughing, rales and bloody expectoration from mouth and nose	Septicemia and bloody exudates in the thoracic and abdominal cavity	30	3.3
18	Sakha	1000	Hybrid	Sneezing, cough nasal discharge and emaciation	Trachitis and pneumonia	20	2
19	Elrhyad	500	Bosuchate	Rales, and ruffled fur	Trachitis and small hemorrhagic spots in lung	3	0.6
20	Dosoque	800	New Zealand	Sneezing, rales and depression	Trachitis and pneumonia	10	1.25
21	Sakha	1000	Hybrid boschute	Dyspnea, cough and nasal discharge	Trachitis and unilateral pneumonia	5	0.5
22	El-Reyad	500	Bosuchate	Bloody mucoid, coughing and nasal discharge	Trachitis hydrothorax consolidation of lung and hepatomegaly	25	5
23	El-Reyad	300	New Zealand	Acular and nasal discharge	Conjunctivitis and trachitis	15	5
24	Foa	1000	Hybrid	Sneezing, depression and abortion	Trachitis, pneumonia and metritis	7	0.7
25	Dosque	500	Bosuchate	Depression, weakness and ruffled fur	Trachitis and pneumonia	3	0.6
26	Dosque	600	Hybrid	Dyspnea, coughing and rales	Trachitis and pneumonia	4	0.6
27	El-Hamel	1000	New Zealand	Cough, sneeze and bloody mucoid discharge	Trachitis, bilateral pneumonia and haemorrhagic septicemia	20	2
28	Qelin	1000	Hybrid	Respiratory sings and abortion in breeder doe	Trachitis, sever congestion in lung, hepatomegally, metritis and urinary bladder distention with bloody urine	50	5
29	Biala	200	New Zealand	Rales, sneezing and cough	Sinusitis, trachitis and bilateral pneumonia	2	1
30	Foa	500	New Zealand	Mucoid discharge, low body weight and ruffed fur	Sinusitis, trachitis and bilateral pneumonia	5	1
31	El-Reyad	500	New Zealand	Sneezing, cough and nasal discharge	Sinusitis, trachitis and hydrothorax	2	0.4
32	Kafrelsheikh	900	New Zealand	Rales, sneezing and mucoid discharge	Sinusitis, trachitis and unilateral pneumonia	3	0.3
33	Dosoque	600	Hybrid	Sneezing, cough, conjunctivitis and nasal discharge	Conjunctivitis, trachitis and pneumonia	2	0.3
34	Qelin	600	Hybrid	Nasal discharge, ocular discharge and sneezing	Conjunctivitis, sinusitis and trachitis	1	0.1
35	Biala	1000	Bosuchate	Mild respiratory signs and sneezing	Sinusitis and trachitis	2	0.2
36	Motobes	600	Hybrid	Dull, depression, decreased body weight and sneezing	Sinusitis and trachitis	2	0.3

37	Sakha	1000	Hybrid	Decreased body weight, cough and sneezing	Trachitis, unilateral pneumonia and liver congestion	1	0.1
38	Foa	500	New Zealand	Depression, ruff fure, in appetite, sneezing and mucoid discharge	Trachitis, unilateral pneumonia	2	0.4
39	Kafrelsheikh	500	New Zealand	Depression, emaciation and mild respiratory signs	Sinusitis, trachitis and pneumonia	1	0.2
40	El-Rhyad	300	New Zealand	Dull, depression and sneezing	Trachitis and pneumonia	2	0.6

## RESULT AND DISCUSSION

Twenty one isolates suspected to be ORT were isolated from 300 diseased rabbits with 7% isolation rate. Examined rabbits were suffered from mild respiratory signs with nasal discharge, depression, ruffled fur, decreased food intake with poor performance, marked dyspnoea and expectoration of blood stained mucous just prior to death. The mortality rate was 0.5 - 5% (Table 2). Similar findings were obtained by *Van Emple et al., (1999)*.

**Table (2):** Mortality rate of examined rabbits suffered from respiratory signs

Farm No.	Mortality%	Result of isolation	Farm No.	Mortality%	Result of isolation
1	6.6 %	-ve	14	2%	-ve
2	0.5%	-ve	15	0.5	+ve
3	1%	+ve	16	2%	-ve
4	0.6%	+ve	17	3.3%	+ve
5	0.4%	-ve	18	2%	-ve
6	3.3%	-ve	19	0.6%	-ve
7	1.6	-ve	20	1.25%	-ve
8	1%	-ve	21	0.5%	-ve
9	0.6%	-ve	22	5%	-ve
10	0.6%	-ve	23	5%	-ve
11	0.75%	+ve	24	0.7%	-ve
12	0.5%	-ve	25	0.6%	+ve
13	1%	-ve	26	0.6%	-ve
27	2%	-ve	34	1.6%	-ve
28	5%	+ve	35	0.2%	-ve
29	1%	-ve	36	0.3%	-ve
30	1%	+ve	37	0.4%	-ve
31	0.4%	-ve	38	0.1%	-ve
32	0.3%	-ve	39	0.2%	+ve
33	0.3%	-ve	40	0.6%	-ve



The main gross lesions in freshly dead and sacrificed rabbits were mild conjunctivitis, mild trachitis, unilateral or bilateral pneumonia with fibrinous exudate in the pleura, pericarditis, peritonitis and petechial haemorrhage on the heart Figs. (1 and 2). Similar findings were reported by *El-Gohary and Awaad (1998)*.



**Fig. (1):** Pneumonia with fibrinous exudate in the pleura



**Fig. (2):** Pericarditis, peritonitis and haemorrhages on the heart

The incidence of ORT isolation from various organs of examined freshly dead and sacrificed rabbits was described in Table (3). The isolation rate was 7% (21 out of 300) from sinus, trachea and lungs, 1% (3 out of 300) from liver and 0% from heart blood. Similar results were obtained by *El- Abasy (2008)* when isolated ORT from internal organs of Muscovy and Balady ducks.

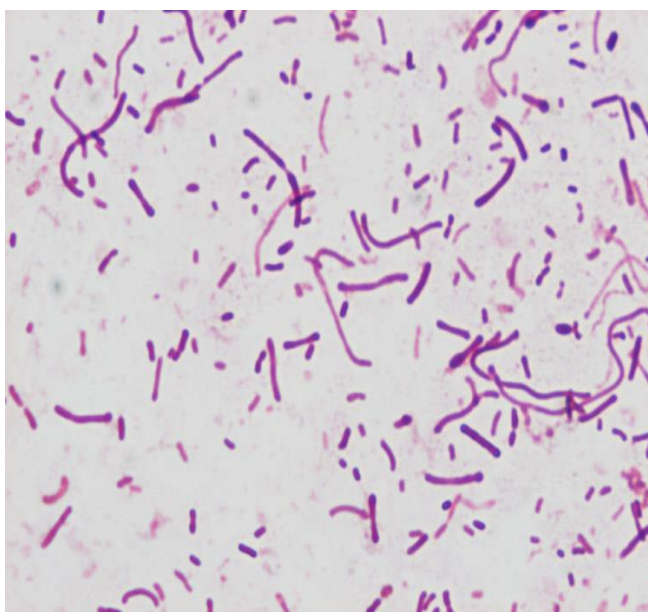
**Table (3):** Prevalance of ORT isolates from various organs of examined rabbits

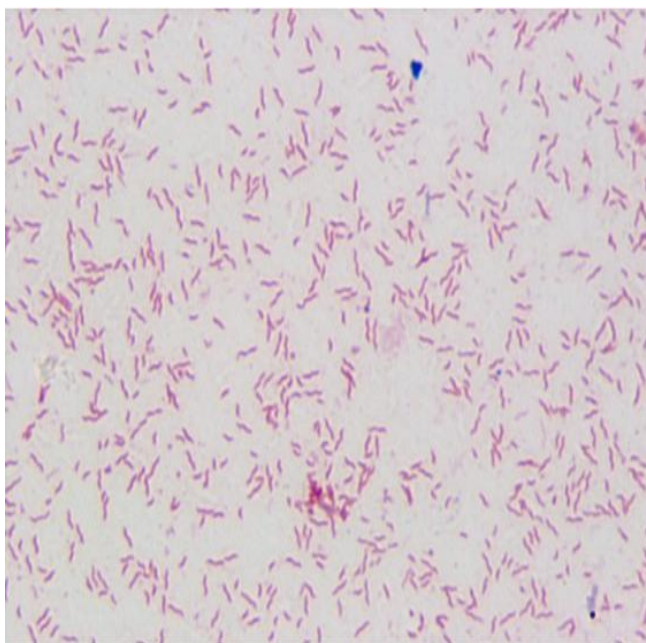
Serial No.	Organs	Examined No.	Positive No.	Isolation rate
1	Sinus	300	21	7%
2	Trachea	300	21	7%
3	Lung	300	21	7%

4	Liver	300	3	1%
5	Heart blood	300	0	0%

Colonies of ORT isolated from sinus, trachea, and liver on sheep blood agar media after 24 hrs incubation at 37° C appeared as pin point but after 48 hrs incubation appeared as pin head circular, opaque, non-haemolytic, grey to greyish white, non-pigmented and convex with smooth surface and entire edges. The colonies have characteristic butyric acid odour.

The bacteria appeared as polymorphic, Gram negative, non-capsulated rods after microscopical examination of Gram's stained film prepared from brain heart infusion broth culture after 24 hrs incubation Fig. (3), or on sheep blood agar media Fig. (4). Similar findings were obtained by *El- Abasy, (2008)* and *Sahar, (2011)*.



**Fig. (3):** Gram negative bacteria isolated on brain heart infusion broth**Fig. (4):** Gram negative bacteria isolated on sheep blood agar

Biochemical identification of the isolated organisms using API 20 NE strip micro test system revealed positive oxidase,  $\beta$ -glucosidase, arginine dehydrate, urease and hydrolysis of  $\beta$  -glucosidase while negative catalase, citrate utilization and. fermentation of glucose, mannitol and arabinose was positive with acid production, while mannose, maltose, carbolic acid and phenile acetic acid were negative. Similar results were obtained by *Sahar, (2011)*.

**Table (4):** The biochemical activity of suspected ORT isolates with API 20 NE

Serial	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
NO3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TRP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GLU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ADH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+



8	11/5/2014	Trachea	untypable
9	11/5/2014	Sinus	A
10	18/5/2014	Sinus	A
11	18/5/2014	Sinus	A
12	18/5/2014	Sinus	Untypable
13	18/5/2014	Sinus	A
14	18/5/2014	Sinus	A
15	18/5/2014	Sinus	A
16	18/5/2014	Lung	A
17	18/5/2014	Lung	A
18	18/5/2014	Lung	A
19	18/5/2014	Trachea	A
20	18/5/2014	Trachea	A
21	18/5/2014	Trachea	A

The antibiogramme of ORT isolated strains was investigated against 19 antimicrobial agents using disc diffusion technique revealed that ORT isolates were sensitive to sulphamethxazol-trimethoprin, , oxytetracyclin, enrofloxacin, ciprofloxacin, amoxicillin, ampicillin, tetracycline, erythromycin and sipramycine but resistance to penicillin, streptomycin, clindamycin, colistine sulphate, lincomycine, gentamycin, vancomycin and neomycin (Table 6). Similar results were reported by *Devriese et al., (2001)*.

**Table (6):** Results of in vitro sensitivity test of ORT isolated strains against 19 antimicrobial agents

Serial No.	Antibacterial agent	Potency of disc (µg)	Standard sensitivity zone (mm)	Zone of inhibition (mm)	S/R
1	Oxytetracyclin	30	14-19	14	S
2	Enrofloxacin	5	10-15	10	S
3	Penicillin G	10	-	-	R
4	Ciprofloxacin	5	15-21	18	S
5	Streptomycin	10	17-22	16	R
6	Clindamycin	2	-	-	R
7	Amoxicillin+clavulanic acid	30	-	10	S
8	Amoxicillin	25	23-31	27	S
9	Ampicillin	10	13-17	15	S

10	Nitrofurantoin	300	-	-	R
11	Colisten sulphate	25	13-18	0	R
12	Tetracyclin	10	14-20	17	S
13	Lincomycin	2	-	0	R
14	Gentamycin	10	12-15	0	R
15	Vancomycin	30	10-18	0	R
16	Erythromycin	30	14-19	14	S
17	Neomycin	30	-	11	R
18	Spiramycin	100	-	16	S
19	Sulphamethoxazole+trimethopriem	25	10-16	16	S

Depression, decreased feed intake, ruffled fur and sneezing with transient nasal discharge were observed in experimentally infected 3-month-old rabbits. Mortality rate was 13 % (Table 7). Post mortem lesions in dead and sacrificed rabbits were trachitis with tracheal exudate, unilateral and bilateral pneumonia with lung congestion, pleuritis, pericarditis, enlarged and congestion liver and spleen. The organism was re-isolated from trachea and lungs. These results are similar to those reported by *Hafez, (1996) El-Gohary and Awaad, (1998) and El- Abasy. (2008)*.

**Table (7):** Lesion score and mortality of ORT experimentally infected rabbits

Group No.	Lesions scores								Mortality No.
	1 <sup>st</sup> week post infection				4 <sup>th</sup> week after infection				
	S	T	Lu	L	S	T	Lu	L	
1A	1	1	1	1	1	1	1	1	1from 5
2A	1	1	1	1	0	0	1	0	1from 5
3A	1	1	1	1	0	0	0	0	0
1B	0	0	0	0	0	0	0	0	0
2B	0	0	0	0	0	0	0	0	0
3B	0	0	0	0	0	0	0	0	0

1A= infected with ORT and untreated. 2A= rabbits infected with ORT and treated with sulpha-.trimethoprim

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3A = infected rabbits with ORT and treated with sulpha-trimethoprim + coconut oil

S = sinus, S0 = no abnormality, S1= watery discharge, S2= mucous discharge and S3 = purulent discharge.

T= trachea, T0 = no abnormality. T1= slight exudate, T2 = moderate exudate and T3= lumen filled with exudate.

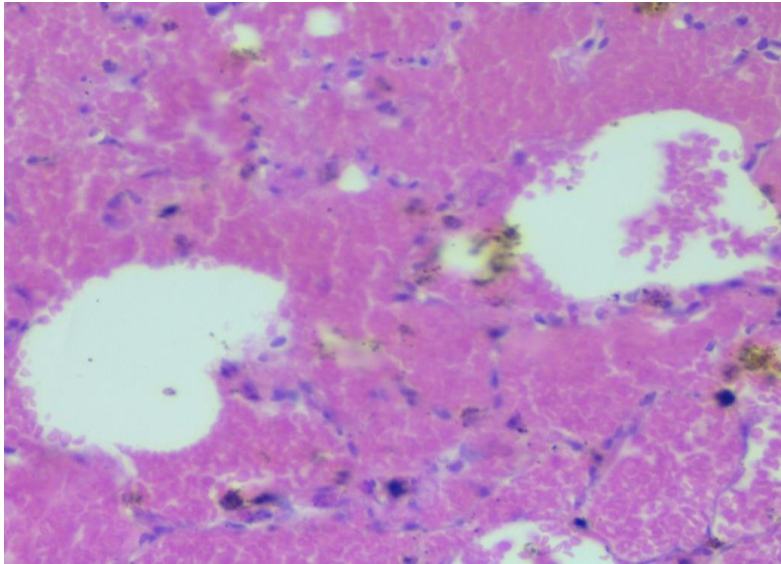
Lu = lung, Lu0 = no abnormality, Lu1= unilateral pneumonia, Lu2 = bilateral pneumonia and Lu3 = consolidation.

L= liver, L0 = no abnormality, L1= congestion, L2 =sever congestion, L3 = consolidation.

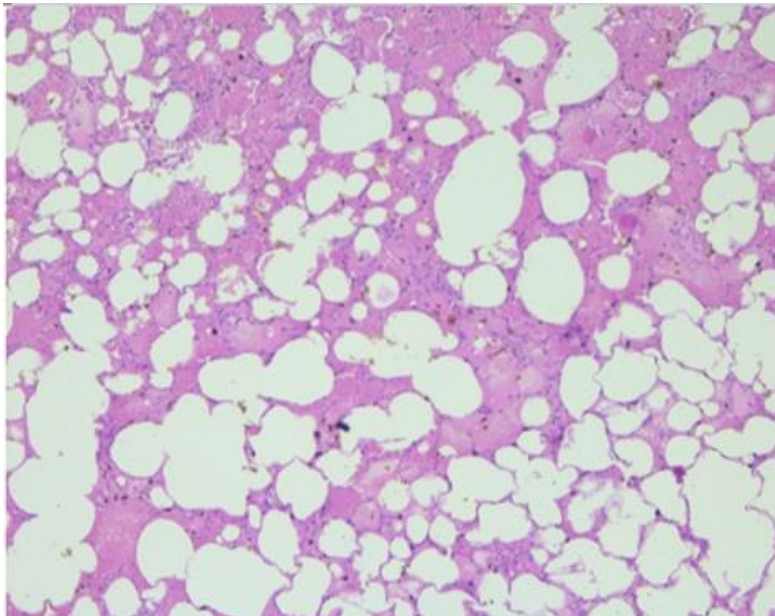
Treatment trials were based on the results in vitro antibiogramme of the isolated ORT organisms. The clinical signs and post-mortem lesions were disappeared in infected rabbits and treated with sulpha-trimethoprine in drinking water for 5 consecutive days with dietary supplementation with coconut oil when compared with infected untreated control group. These results were similar to that reported by *Shihata and Ibrahim, (2004) and El-Abasy, (2008)*.

Histopathological examination of lungs in ORT infected untreated rabbits revealed fibrinous pneumonia, emphysema and haemorrhage (Fig 5). Lungs of ORT infected rabbits and treated with sulpha-trimethoprim plus coconut oil showed marked improvement as shown in (Fig. 6) while control group shown no pathognomonic lesions (Fig.7).



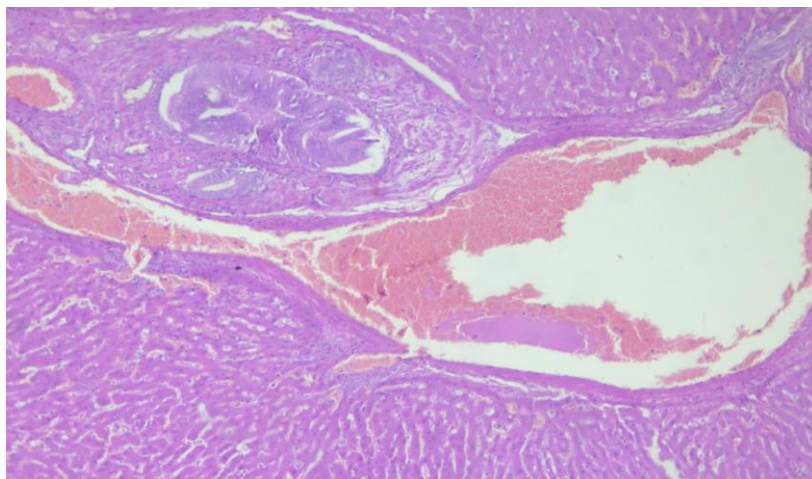


**Fig. (5):** lung of ORT infected untreated rabbits showed fibrinous pneumonia, emphysema and haemorrhage.

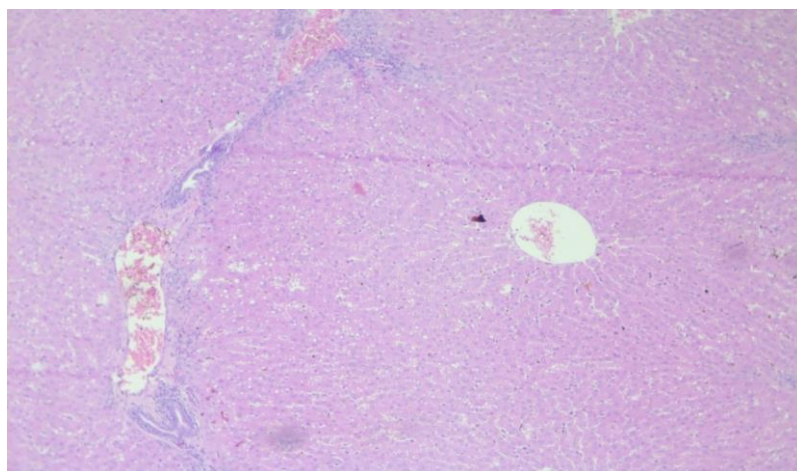


**Fig. (6):** lung of ORT infected rabbits and treated showed mild fibrinous pneumonia and mild congestion

Microscopical examination of liver of ORT infected untreated rabbits revealed severe lesions as thrombus, haemorrhage and inflammatory cell infiltration Fig (7). Sulpha--trimethoprim + coconut oil treatment of infected rabbits revealed improvement in liver lesions Fig (8).



**Fig. (7):** liver of infected untreated rabbits showing thrombus, haemorrhage and inflammatory cells infiltration.



**Fig. (8):** Liver of infected and treated rabbits showing mild congestion and mild inflammatory cells infiltration.

## CONCLUSION

ORT is claimed to be a new pathogen isolated from diseased rabbits in Kafrelshikh Governorate, Egypt. Further studies on the pathogenicity of single and mixed infection and trials for production of potent vaccine for protection of rabbits against ORT infection are needed.

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