

INCIDENCE OF PATHOGENIC *ESCHESICHIA COLI* IN SOME POULTRY SPECIES IN EGYPT AND ITS EFFECT ON CHICKENS PERFORMANCE

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ABSTRACT

The highest incidence of positive pathogenic E.COLI (APEC) isolated in this study was from chickens (57.96%) followed by ducks ,pigon, turkey ,ibis and quail with a percentage ,25.48%, 5.10%, 4.46%, 3.82% and 3.18% respectively. Heart , liver and lungs were the important tissues in production of APEC E.COLI O26 , O127, O55, O119, O111, O78, O86, O2 and O114 were the most prevalent serogroups identified in this study .using PCR five virulence associated genes in the APEC serogroups were irp2 , iron, ompt, iss and hlyf from all examined isolates . xperimental infection of broiler chickens with the different isolated E.coli serogroups ,o78 and o2 showed the highest mortality rates 60% and 50% respectively with severe reduction in the mean body weight from 20-30% . Antibiotic sensitivity of the isolated APEC was studied.

INTRODUCTION

The association of *Escherichia coli* strains with disease conditions in avian species was recognized over a century ago, but these strains were never accorded a special status (*Qadri et al., 1994*). However, lesions in which *E. coli* is the primary and often the secondary agent

cause economic damage due to lower corporal development, insufficient feed conversion, increasing mortality, higher cost with medicine, and condemnation of carcasses (**Brasil, 2006**).

E. coli strains causing systemic disease in poultry (avian colibacillosis) are termed avian pathogenic *E. coli* (APEC). Colibacillosis is a disease of severe economic significance to all poultry producers worldwide and is characterized by a diverse array of lesions. The most common lesions associated with colibacillosis are perihepatitis, airsacculitis and pericarditis, although other syndromes such as egg peritonitis, salpingitis, coligranuloma, omphlitis, cellulitis and osteomyelitis, arthritis may be encountered (**Gomis et al., 2001**).

APEC strains fall under the category of extraintestinal pathogenic *E. coli*, which are characterized by the possession of virulence factors that enable to live extraintestinal life. These virulent factors have been identified (**Dozois et al., 2000; Johnson et al., 2006**). However, no specific virulent factor that contributes entirely to the pathogenicity of APEC has been discovered (**Province and Curtis, 1994**). Multiplex PCR was proved that virulent strains which are already typed as APEC had 5 to 8 virulence-associated genes but avirulent strains which are already typed as non-APEC strains had at most 4 virulence-associated genes (**Ewers et al., 2005**).

Thus the aim of this study was to detect APEC from some poultry species (chickens, ducks, turkeys, pigeons, quail and ibis)using different diagnostic tools including polymerase chain reaction (PCR). In addition, the sensitivity of the confirmed APEC strains to various antibiotics used in Egypt, as well as the pathogenicity of some isolated serotypes for chickens were discussed.

MATERIAL AND METHODS

Sampling:

One hundred broiler chickens, 50 ducks, 10 from each of turkey, pigeon, quail, and ibis (moribund and freshly dead) were collected from different poultry farms in Sharkia governorate, Egypt. The signs were mainly respiratory signs (coughing, sneezing, mucoid nasal secretion and gasping) and swollen head accompanied with enteritis and emaciation. Nine hundred and fifty samples from liver, lungs, air sacs, intestine and heart blood were collected for bacteriological examination (500 sample from chickens, 250 sample from ducks and 50 sample from each of the examined other poultry species).

Bacteriological examination:

Tissue samples were plated on MacConkey agar plates (Difco, Detroit, MI, USA). The lactose fermenting colonies were reinoculated to Eosin Methylene Blue agar plates (Difco, Detroit, MI, USA). Metallic sheen colonies were transferred to Nutrient agar slants and incubated at 37° C for 24 h and then stored at 4° C for further identification. Identification of isolates was done according to *Cloud et al., (1985)*.

Serodiagnosis of *E.coli*:

The confirmed *E. coli* isolates were serologically identified according to *Kok et al., (1996)* by using rapid diagnostic *E.coli* antisera sets (Difco, Detroit, MI, USA) for diagnosis of the Enteropathogenic types.

DNA preparation and Detection of virulence factors by Polymerase Chain Reaction (PCR):

Each of glycerol stock serodiagnosed *E. coli* was incubated in Nutrient broth (Difco) and grown overnight in shaking incubator at 37° C. The enriched medium in a 1.5 ml-eppendorf tube was microcentrifuged in 1,500 rpm for 10 min, and the supernatant was discarded. Autoclaved distilled water (DW) or TE buffer (10 mM Tris-HCl, 1 mM EDTA at pH 8.0) was added to the pellet, Then freezed for 10 min at -80° C, and incubated (boiled) for 5 min at 95° C. After repeating this procedure 3 to 4 times, and then centrifuged again in 1,500 rpm for 10 min. DNA concentration in supernatant was evaluated by Nanodrop (ND-1000, Nanodrop Technologies, Wilmington, DE, USA).

Tested *E. coli* were examined for *astA*, *irp2*, *iroN*, *ompT*, *iss*, *hlyF*, *IutA* and *tsh* virulent genes using PCR. The PCR procedure was applied after DNA extraction, according to the protocol described by **Boom et al., (1990)**.

Antibiogram:

Antibiotic sensitivity test was performed according to the procedures of **Aerts et al. (1995)**.

Experimental infection:

One hundred, two weeks old Ross chicks were divided into 10 groups table (1)

Table (1): Experimental design for Experimental infection)

Groups	Number	Serotypes E coli	Dose	Route	Treatment	Re isolation
1	10	O26	1*108 Cfu Former et al., 1994	I/M	Norfloxacin 10mg/kg for 3-5 days after onset of symptoms	From faeces at 28, 35 and 42 days old
2	10	O27				
3	10	O111				
4	10	O119				
5	10	O86				
6	10	O2				
7	10	O78				
8	10	O114				
9	10	O55				
10 control	10	-	-	-	-	-

Infection in all groups at 21 day of age, BW, FL and FCR were estimated at 28,35 and 42 days old.

RESULTS

Heart blood was reported to have the highest incidence of the biochemically identified *E. coli* isolates in all species examined in our study. Lungs came second to heart followed by liver, spleen and air sacs. The total percentage of *E. coli* isolates in these tissues in all species examined were 31.85%; 22.93%; 18.47%; 14.01% and 12.74% respectively (Table2).

The highest incidence of positive *E. coli* isolates was recorded to be in chicken (57.96%), followed by ducks (25.48%), pigeon (5.10%), turkey (4.46%), ibis (3.82%) and quail (3.18%) as shown in Table2. This result is considered reasonable and parallel with the high numbers of chicken farms in Egypt compared with other poultry species.

Interestingly, Ibis which is usually observed flying around poultry farms in Egypt all over the year, found to harbor avian pathogenic *E. coli* (Table2).

Table (2): Incidence of positive E.COLI isolates and serogroups from tissues of different avian species

Species	+ve E.COLI samples biochemically						+veE.COLI											
	+veE.COLI	liver	H.bl	lung	A.S	spl	O26	O127	O111	O119	O86	O2	O78	O114	O55	TO.1	UNT	TO.2
ch	57.96%	19(20%)	31.9%	25.3%	13.2%	8.8%	17	18	0	16	0	0	12	0	26	89	2	91
D	25.48%	6(15%)	25.5%	20%	15%	22.5%	7	8	13	0	11	0	0	0	0	39	1	40
T	4.46%	1(14.3%)	42.9%	28.6%	0%	14.3%	2	1	0	0	0	2	0	0	0	5	2	7
p	5.10%	2(25%)	37.5%	12.5%	12.5%	12.5%	4	3	0	0	0	0	0	0	0	7	1	8
ib	3.82%	0(0%)	33.3%	16.7%	16.7%	33.3%	2	1	0	0	0	0	0	2	0	5	1	6
Q	3.18%	1(20%)	40%	20%	0%	20%	1	2	0	0	0	0	0	0	0	3	2	5
Total	157 (100%)	29 (18.47%)	50 (31.85%)	36 (22.93%)	20 (12.74%)	22 (14.01%)												

Total² refers to total number of both identified and untyped *E. coli* serogroups

TO1=TOTAL1

H.bl=heart blood

TO2=TOTAL2

A.S=air sacs

UNT=UNTYPED

Spl=spleen

The majority of APEC strains (94.3%) were typeable with standard O antisera; from the typeable isolates (148), a total of 9 different O serogroups were identified (Table2). The most prevalent serogroups in different examined poultry species were O26 (21%), O127 (19.7%), O55 (16.6%), O119 (11.5%), O111 (8.3%), O78 (7.6%), O86 (7%), finally both of O2 and O114 (1.3%).

Our PCR results confirmed that all identified *E. coli* serotypes were classified to belong to APEC, Patterns and combinations of virulence-associated genes for 9 APEC strains isolated in the present study are summarized in Fig. 1 and Table3. Although the specimen origins of APEC strains were diverse, the existence of virulence-associated genes had similar patterns (Table3, Fig. 1).

Table (3): Distribution of virulent genes in the serogroups of *E. coli* isolates used in the PCR analysis

Virulent genes	O86	O127	O114	O26	O78	O119	O111	O55	O2
<i>astA</i>	+	+	+	-	+	+	-	+	+
<i>Irp2</i>	+	+	+	+	+	+	+	+	+
<i>Iron</i>	+	+	+	+	+	+	+	+	+
<i>ompT</i>	+	+	+	+	+	+	+	+	+
<i>Iss</i>	+	+	+	+	+	+	+	+	+
<i>hlyF</i>	+	+	+	+	+	+	+	+	+
<i>IutA</i>	+	±	±	+	+	-	-	-	+
<i>Tsh</i>	+	±	±	+	+	+	-	-	+

On average, detection rate of *Irp2*, *iron*, *ompT*, *iss* and *hly* genes showed that they existed in APEC strains with 100% (Table3, Fig. 1).

Our data showed that the identified serogroups from different poultry species showed a marked sensitivity to newly used antibiotics in Egypt like cefotaxin and Norfloxacin and variable degrees of sensitivity to other antibiotics as shown in table 4.

Table (4): Antibiogram of various pathogenic *E. coli* serogroups isolated from examined poultry species towards various antibiotics

Antibiotic		O26	O127	O111	O119	O86	O2	O78	O114	O55
Cefotaxin (CTX)	30 mg	+++	++++	+++	++++	++++	+++	++	+++	+++
Norfloxacin (NOR)	10 mg	+++	++	++	+++	++	++	+++	++++	+
Ciprofloxacin (CIP)	5 ug	+	+	+++	+++	+++	++++	+++	+++	+
Danofloxacin (DAN)	5 mg	++++	+	++	+++	++	++++	++	++++	+
Streptomycin (S)	10 mg	++	+	+	++	++	+++	+	+	++
Azithromycin (AT)	15 mg	-	+++	+	+++	++	++	+++	++	++
Amoxicilline (AX)	25 mg	++++	-	++++	+	+++	+++	+	++	+
Florfenicol (FFC)	30 mg	++++	+	+	-	+	+++	++	+++	++++

(++++) Highly sensitive, (+++) Moderately sensitive, (++) Sensitive, (+) Weakly sensitive, (-) Nonsensitive.

Ross chickens were experimentally infected with the different isolated E.coli serotypes. The mortality rates of this experimental infection were clarified in (Table5). It was shown that E.coli O 78 and O2 had the highest mortality rates with percentages of 60% and 50% respectively, while E.coli O111 and O55 had the least mortality rates in Ross chickens with percentages of 10% for each. Other E.coli serogroups had variable effects on the experimentally infected chickens as E.coli mortality pattern was 40, 30 and 60% due to infection with O86, O119 and O26 respectively. Both of O127 and O114 led to 20% mortality among their infected groups (Table5). These results were considered reasonable as they were parallel with the harboring of the virulence associated genes, as E.coli O2, O78 and O86 had 100% of the examined virulent factors, While E.coli O55 and O111 had only 6 and 5 out of 8 tested virulence associated genes (Figure 1 and Table3

Experimental infection with different E.coli serotypes in Ross chicken led to a significant decrease in mean body weight for all infected groups (1-9) groups, at 4th week (before treatment).

E.coli O2 had the lowest mean body weight 705.221 ± 29.547 compared with the control 1090.55 ± 10.883 . Other E.coli serotypes led to severe reduction in the mean body weight about 20-30% reduction (Table5). This reduction was continuous till 6th week of the experiment compared with the control, although much improved after treatment (Table5). Our results correspond with the findings of *Hui et al., (2002)* and *Oh et al., (2011)*.

Table (5): Effect of E coli infection on mean body weight, feed intake and feed conversion ratio ,3,4 and 5 weeks post infection

Group	1st	2nd	3 rd	4th	5th	6th	7th	8th	9th	p10th	
E.coli serology	O26	O127	O111	O119	O86	O2	O78	O114	O55	Control	
No. of birds	10	10	10	10	10	10	10	10	10	10	
Mortality %	30	20	10	30	40	50	60	20	10	0	
Re isolation	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	
Mean body weight*	28 day	813.25 bc ± 31.473	814.626 b ± 32.423	814.26 bc ±36.211	829.87 b ±27.008	825.48 b ±13.53	705.221 c ±29.547	737.33 bc ±28.715	744.825 bc ±26.496	890.45 b ±12.883	1090.55 a ±10.883
	35 day	1033.25c ±19.811	1096.6c ±21.753	1058.1c ±25.578	1136.625b ±30.82	1119.9b±1 1.003	1003.3c ±17.05	1018.0c ±37.29	1051.57c± 2.707	1315.7b±2 2.4535	1515.7a ±12.4535
	42 day	1307.35c ±18.088	1386.967c ±19.885	1372.1c ±21.952	1550.625b ±26.403	1507.14b± 12.365	1319.14c ±16.862	1370.2c±2 7.32	1547.45b± 24.703	1608.4b±1 2.371	1908.4a ±12.371
Feed intake **	28 day	640.0b ±9.0711	659.0b ±42.7735	638.0b ±15.7735	655.0b ±7.9511	648.0b±17 .0711	602.0c ±18.160	600.0c ±15.7735	650.0b±5. 7735	650.0b ±17.0711	750.0a ±15.6195
	35 day	748. 0b ±17.0711	738. 03b ±10.5774	728. 03b ±10.5774	740. 0b ± 24.0825	687.5c±10 .4082	680.0c ±28.1650	752.0b ±15.7735	748.3b±10 .5774	740.0b ±17.0711	853.0a ±15.6195
	42 day	859.0b ±17.0711	847.0b ±20.8167	860.0b ±20.8163	850.0b ±24.0825	840.0b±26 .3299	817.0c ±18.1650	805.0c ±25.7735	847.0b±20 .8160	850.0b ±27.0711	880.0a ±15.6195
FCR***	28 day	2.92b ±0.20707	2.81b ±0.1577	2.98b ±0.205774	2.98b ±0.13707	3.38c ±0.10707	4.35d ±0.2488	3.55c ±0.10577	2.90b±0.2 0577	2.83b ±0.10707	1.82a ±0.20743
	35 day	2.35a,b ±0.00707	2.51b ±0.20577	2.56b ±0.10577	2.44b ±0.20408	2.55b ±0.10408	2.81c ±0.00816	2.79c ±0.00577	2.82c±0.0 0577	2.54b ±0.120707	2.189a ±0.206588
	42 day	2.37a ±0.00707	2.49a ±0.00577	2.40a ±0.00577	2.29a ±0.00408	2.39a ±0.00408	2.29a ±0.00816	2.27a ±0.00577	2.19a±0.0 060	2.29a ±0.00707	2.47a ±0.151088

*=mean body weight (gram) post 3,4 and 5weeks

= feed intake (gram)**

***=feed conversion ratio (FCR)post 3,4 and 5 weeks

Sign. (P<0.05)

DISCUSSION

The highest incidence of positive *E. coli* isolates was recorded to be in chicken (57.96%), followed by ducks (25.48%), pigeon (5.10%), turkey (4.46%), ibis (3.82%) and quail (3.18%). This result is considered reasonable and parallel with the high numbers of chicken farms in Egypt compared with other poultry species.

This result goes in correspondence with *Xi et al., (2007)*, who recorded 6 cases of septicemia in crested ibis (*Nipponia nippon*), in China, *E. coli* was also reported to be the cause of sudden death and severe diarrhea in these cases.

The distribution of *E. coli* in different tissues of poultry species corresponds with *Youssif et al., (2008)* who reported that the highest incidence of *E. coli* isolates was observed in the heart followed by liver and finally air sacs in broiler and laying chickens distributed in Ismailia province, Egypt. Internationally, similar pattern of tissue distribution of *E. coli* was observed in Japanese quails (*Coturnix coturnix japonica*) experimentally infected with the *Escherichia coli* serogroups O2 isolated from a turkey (*Nain and Smits, 2011*).

The majority of APEC strains (94.3%) were typeable with standard O antisera; from the typeable isolates (148), a total of 9 different O serogroups were identified. The most prevalent serogroups in different examined poultry species were O26 (21%), O127 (19.7%), O55 (16.6%), O119 (11.5%), O111 (8.3%), O78 (7.6%), O86 (7%), finally both of O2 and O114 (1.3%). These results go in agreement with *Wang et al., (2010)* who reported that *E. coli* O26, O78 and O2 were from the most prevalent *E. coli* strains isolated from broiler chickens found to have avian colibacillosis in Guangdog, south of China between 2005 and 2008. In Sharkia governorate, Egypt, *E. coli* O55, O78 were also detected by (*Osman, 1992*) .*E. coli* O126, O55 ,O8, O78,O114, O44 recovered by (*Taha, 2002*). In regard to other poultry species, our results partially correspond with *Hegazy (1992)*, who isolated *E. coli* O128, O126 and O114 from 3 flocks of ducks. *E. coli* O26 and O119 were previously detected in Mansura city, Dakahlia governorate (*El-Sayed et al., 2002*).

Our PCR results confirmed that all identified E. coli serotypes were classified to belong to APEC, Patterns and combinations of virulence-associated genes for 9 APEC strains .Although the specimen origins of APEC strains were diverse, the existence of virulence-associated genes had similar patterns.

On average, detection rate of *Irp2*, *iron*, *ompT*, *iss* and *hly* genes showed that they existed in APEC strains with 100%. However, *astA*, *IutA* and *tsh* genes were less frequently detected in the identified serogroups. These genes were also found in colibacillosis isolates from other countries (*Janßen et al., 2001; Delicato et al., 2003; Rodriguez-Siek et al., 2005*), who revealed that multiple potential virulence genes may participate in the pathogenesis of colibacillosis. For instance, other studies showed that the *tsh* was detected in 19%, 49.7%, 84%, and 85.3% of the E. coli strains, respectively (*Dozois et al., 2000; Janßen et al., 2001; de Brito et al., 2003; Zhao et al., 2005*), whereas in about 75% of the strains in this study and in 39.5% of the colibacillosis strains (*Delicato et al., 2003*). Similarly, the *iss* gene was detected in 83%, 86%, and 38.5% of the colibacillosis strains (*de Brito et al., 2003; Delicato et al., 2003; Zhao et al., 2005*), whereas it was found in 100% of the isolates in this study.

The difference in such associations may be related to virulence-associated genes, geographical origin, and antimicrobial use of the strains under investigation. Thus different APEC strains can exploit several alternative paths to adhere, colonize, and invade their hosts .In regard to the isolated serogroups, we observed that E.coli O2, O78 and

O86 had 100% of all tested virulence related genes. This finding goes in line with *Vidotto et al., (1990)* who mentioned that avian pathogenic E.coli most commonly belongs to O1, O2, or O78 and typically possesses virulence factors such as lipopolysaccharide, temperature-sensitive hemagglutination (Tsh), and increased serum survival factor (ISS).

Antimicrobial therapy is one of the important measures for reducing significant economic losses to the poultry industry caused by colibacillosis.

In our study, sensitivity test showed that O26 and O127, which were the majors serotypes isolated from turkey, ibis and quail were weakly sensitive to ciprofloxacin.this result corresponds with *Mueller-Doblies et al., (2013)* who recorded ciprofloxacin resistant E. COLI isolates in turkey hatcheries in the United Kingdom.

This finding goes in line with *Vidotto et al., (1990)* who mentioned that avian pathogenic E.coli most commonly belongs to O1, O2,or O78 and typically possesses virulence factors such as lipopolysaccharide, temperature- sensitive hemagglutination (tsh), and increased serum survival factor (ISS).

In conclusion, experimental infection of Ross chicken with different E.coli serotypes led to significant reduction in the mean feed intake and body weight and increase in the feed conversion ratio. The worst effects were recorded in case of E.coli and O87. These results were considered reasonable due to these two serotypes harboring 100% of the virulent factors tested in our study.

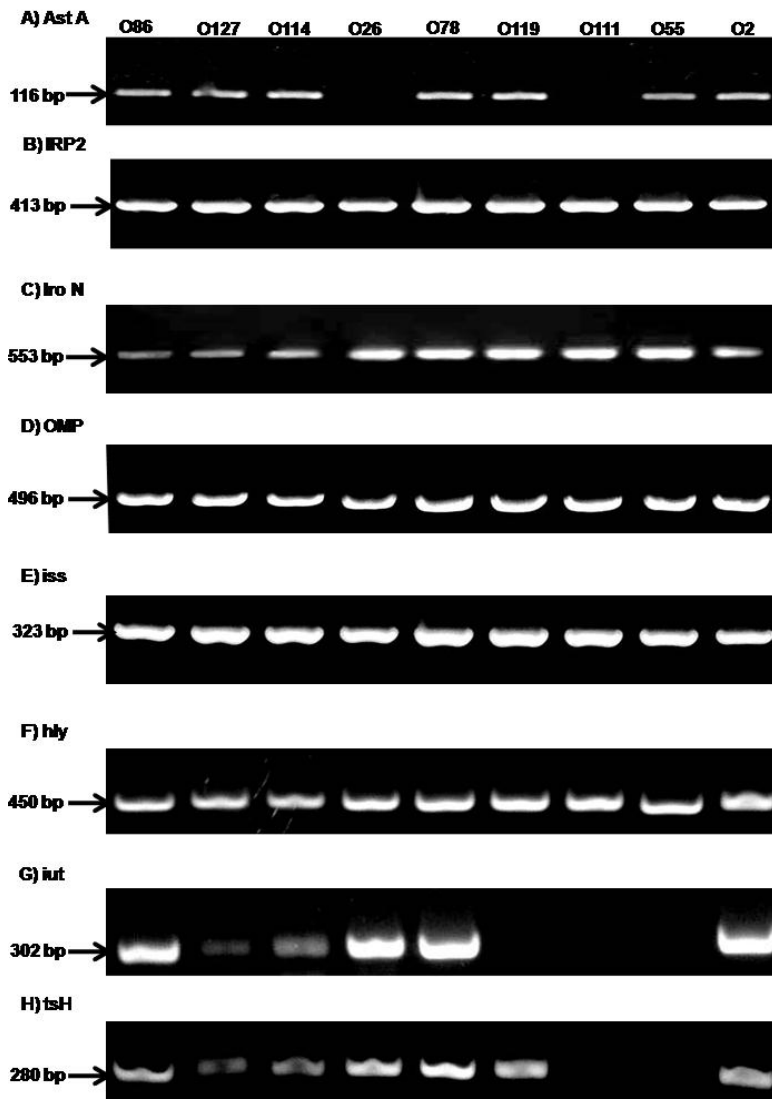


Fig. (1): Amplicon patterns of virulence-associated genes by PCR.

DNA bands for: A) *astA*; B) *irp2* ;C) *iroN*; D) *ompT*; E) *iss*; F) *hlyF*; G) *iutA*; H) *tsh* in the different serotypes of *E. coli* isolated from different poultry species. The PCR conditions were declared in the material and methods section. The marker size (bp) was declared with arrow beside the referred band

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نسبة حدوث مكروب القولوني الممرض في بعض سلالات الدواجن في مصر وتأثيرها علي أداء الدجاج

بنسب مرتفعة (%57096 من الدجاج والبط والحمام والرومي وابو قردان والسمان) (APEC) تم عزل الميكروب القولوني الممرض و (%25048، %501، %4046، %3082، %3018) علي التوالي و كانت نسبة العزل من القلب والرئتين والكبد مرتفعة وتم تصنيفها سيولوجيا فكانت كالآتي:

باستخدام اختبار البلمرة المتسلل وجد خمس جينات ضارية ومصاحبة لـ O26, O127, O55, O119, O111, O78, O86, O2, O114 . بالعدوى الصناعية بهذه المعزولات في الدجاج والسمان كان hlyf , iron, ompT , irp2 . وهي نسبة وفيات عالية %50-60 علي التوالي مع انخفاض شديد في متوسط الأوزان من 20%-30% أعطوا O2,O78 كما تم دراسة حساسية هذه للمضادات الحيوية المختلفة.