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EPIDEMIOLOGY OF *AERMONAS HYDROPHILA* INFECTION IN DUCKS TRANSMITTED FROM FISH IN DUCK – FISH FARM

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ABSTRACT

This study was carried out to concise the pathological relationship, which may occur between ducks and fish in the integrating duck-fish-farms. Duckling 21- day-old were experimentally infected with *Aeromonas hydrophila (A. hydrophila)*, which was previously isolated from fish. The infection was induced by different routes; Intraperitoneally, intramuscularly, subcutaneously and orally. Other three additional groups were kept in contact with experimentally infected groups (in the same pens). The symptoms, post mortem lesions and mortality rate differed according to the route of infection. The mortality rate ranged from 40% (in intraperitoneal infection) to 0% (in oral infected and contact groups). Reisolation of *A.hydrophila* could be achieved from different internal organs of dead birds but in varying percentages. Shedding rate of *A.hydrophila* in droppings was studied, all infected and contact groups, shed the *A.hydrophila* but at different times post infection. The in-vitro sensitivity test, revealed that *A.hydrophila* was sensitive to many antibiotics, like kanamycine and tetracycline.

INTRODUCTION

The integrating systems of ducks fish farming are spreading nowadays in Egypt for their benefits exchange. The fish is highly nutritive source for ducks. The ducks are known as living manuring machine as their droppings form a very good source of fertilizer in fish ponds. Besides manuring , ducks eradicate the undesired insects, snail and their larvae, which may act as vectors of fish pathogenic organisms (**Santhanan *et al.*, 1987**). In addition, the fish farmer in case of duck - fish culture obtain free pond fertilization, there by reducing their operating costs, and the duck owners receive rent - free land and free pond water for duck husbandry purposes (**Saleh, 1990**).

One of the most important drawback of such system were that fish can infect ducks with many pathogens causing severe problems (**Ghith, 1995 and**

Mustafa, 1996). Therefore this work was carried out to trace back the epidemiology of *A.hydrophila* infection in ducks transmitted from fish.

MATERIAL AND METHODS

Fish samples:

Fish samples were collected from a septicemic outbreak of Tilapia fish in a private fish farm. The fish were suffering, from severe clinical signs including congestion, redness of the fins, skin alteration, abdominal ascitis, unthriftness, loss of balance, detachment of the scales and haemorrhagic patches on the outer surface.

The most common postmortem (P.M) lesions were congested liver with focal hemorrhages, distended gall bladder, congested and enlarged spleen, the kidneys also were congested and enlarged accompanied with enteritis intestinal inflamation. Samples from these different organs were collected for A.hydrophila isolation according **Popoff and Veron, (1976)**. The clinical examination and postmortem examination was performed according to **Plumb, (1994)**.

Ducklings:

Ninety-five-21-days old Muscovy ducklings from commercial hatchery were used for experimental infection.

Cultural media and reagents:

a) Media for isolation:

- Nutrient broth (oxoid).
- Nutrient agar (oxoid).
- Rimler shotts agar (R.S) (**Shotts and Rimler, 1973**).
- Mac Conkey agar (oxoid).

b) Media for identification:

- Semi - solid nutrient agar used according to **Quinn *et al.* (1994)**.
- Sugar media 1% (glucose, lactose, inositol, L-arabinose, sucrose, salicin , maltose and mannitol).

c) Cytochrom oxidase reagent used for C.O test according to **Finegold and Martin, (1982)**.

c) Gram's stain.

Antibiotic Sensitivity test:

The sensitivity of *A. hydrophila* to different antibiotics was carried out using the disc diffusion technique according to **Lennette *et al.*, (1980) and Collins *et al.*, (1989)**.

Experimental designs:

Nine groups of ducks, ten birds each were used, aged 21 days old (recommended age for rearing ducklings in fish ponds). Before artificial infection, random samples from five ducklings scarified and subjected to post mortem and bacteriological examinations. These samples proved to be negative for *A. hydrophila*. The routes of infection were orally and subcutaneously (S/C) (resembling natural routes of infection) in addition to intramuscular (I/M) and intrapretoneal (I/P) injection. The injecting dose was 0.5 ml of 4.3×10^8 viable cells / ml broth (**Ghith, 1995**). Other groups were kept in contact to the infected groups (in the same pen with marking). The different groups were as the following.

G1	: infected orally	G2	: contact to G1
G3	: infected S/C	G4	: contact to G3
G5	: infected I/M	G6:	contact to G5
G7	: infected I / P	G8:	contact to G7
G9	: non-infected control		

All infected groups were kept separately for 21 day post infection under observation for recording clinical signs, mortalities and P.M lesions, the contact birds kept in the same pens after marking. Cloacal swabs were collected at 0,1,2,3,4,7,10,14&21 day post infection for estimating bacterial shedding rate. The dead birds were subjected to P.M examination and lesions were recorded. Reisolation of *A. hydrophila* were tried from different organs of dead or sacrificed (control) birds

RESULTS

Aeromonas hydrophila isolation and identification:

A virulent *A.hydrophila* isolates were recorded from liver, spleen, kidneys and at more higher extent from skin and fin lesions of naturally infected fishes using broth, MacConky's agar and Rimler shotts agar. Where examination of growth colonies revealed typical *A.hydrophila*, round, dome - shaped yellow colonies on R. S. media. Smear from separate colony was stained with Gram's stain, proved to be negative gram stain. The organism was examined using a semisolid agar which proved its motility.

The biochemical identification:

Cytochrom oxidase test, which differentiate between aeromonas and family enterobacteriacae especially citrobacter organism, which gives yellow colonies on R.S. medium. This test give positive reaction when development of deep blue or purple color within 1seconed (culture should not be older than 24 hours to avoid false result).

The different previously mentioned biochemical tests gave positive results except lactose and inositol which gave negative reaction. From the previously mentioned results the isolated organism proved to be *A.hydrophila*

Results of experimentally infected ducklings:

Infected ducklings showed, off food, ruffled feather and increased water consumption and diarrhea. These symptoms more obvious in I/P, I/M and S/C infected groups. In oral infected groups, however the signs were less severe. The mortality rate was higher in I/P(40%) then in I/M (30%) and S/C (20%) but no mortalities were recorded in oral infected and contact groups as shown in Table (1) and Fig.(1) .

The most important postmortem lesions were congested blood vessels, congested liver with streaks and patches of hemorrhages, congested intestine with enteritis and nephritis the ureters were distended with urates as shown in photos. (1-6).

The contact birds showed some ruffling feather and dull expression without mortalities the results of reisolation of A.H from different organs of infected dead birds were varied according route of infection and organs samples as shown in Table (2) and Fig (2). The results of cloacal swabs reisolation revealed detection of *A. hydrophila* in dropping of infected and contact birds for different periods post -artificial infection as shown in Table (3) and Fig.(3). The results of in vitro sensitivity test of *A. hydrophila.* showed that it was highly sensitive (+++) for kanamycine, streptomycin, tetracycline, lincospectin, neomycin and oxytetracycline, moderaely sensitive (++) to ampicilline, chloromphenicol, amoxycilline and nalidixic acid and weakly sensitive (+) to erythromycin and nitrofurantion as showen in Table (4).

DISCUSSION

This investigation demonstrated the effect of one of the most pathogenic microorganism; *A.hydrofila* of naturally infected fish (**Mustafa, 1996**) on the health of ducklings. The isolation of Aeromonas species from naturally infected fish was previously recorded by **Lewis, (1973); Khan *et al.*, (1981); Easa *et al.*, (1985); Faisal *et al.*, (1989) and Lehane and Rawlin, (2000)**.

Regarding the pathogenicity of *A.hydrophila* to 21–day-old ducklings. The mortality rate was high following the intraperitoneal route of infection (40%), i/m (30%) s/c route (20%) but oral route (0%) as shown in Table (1) and Fig. (1) which disagreed with **Shane and Gifford, (1985) and Ghith, (1995)**, who stated that ducklings were resistant to s/c infection, whereas infection via yolk sac with *A. hydrophila.* led to 100% mortality rate, this is at one day old of infection . On the other hand the results agreed with that of **Mustafa, (1996)**. This controversy may be attributed to use of different

isolates of *A. hydrophila* and using different age of ducks, where age resistance might be acquired. Oral infection of 21-day-old ducklings revealed only general signs like ruffling feathers, dullness and diarrhea without any mortalities. This may be contributed to many factors including difficulty of absorption or penetration of organism through intestinal wall, the effect of gastrointestinal secretions or enzymes and dilution of the organisms.

Regarding P.M. finding of dead ducklings, liver and intestine were congested with hemorrhagic patches and enteritis, also nephritis were recorded, these are consistent with findings of **Bisgaard, (1981); Glunder, (1989); Ocholi and kalejaiye, (1990) and Mustafa, (1996).**

The contact ducklings shows the same general symptoms but in less degree, which indicated that the ducklings may get latent infection due to their contact with infected birds within their environmental. This is also confirmed by reisolation of *A. hydrophila.* from cloacal swabs of infected and contact birds for many days as showing in Table (3) and Fig(3).This indicated that contact birds got latent infection and became carrier and disseminated the organism in their dropping and contaminating the surrounding environment . **Shan and Gifford, (1985)** explained that the infection was properly by fecal shedding of *A. hydrophila.* which could persist in water and moist soil. In addition of that *A. hydrophila.* can resist low temperature 10^0 C and grow well than at 30^0 C (**Schubert and Matzinou, 1990**).

The infected birds shedded *A. hydrophila.* in droppings for 14d. in I/P infected group (by77.5%) and I/M infected group (by 60%) and for 7d. in S/C infected group (by40%) and orally infected (by35%). These results were previously declared by **Shane and Gifford, (1985)** who proved that A.*hydrophila* shedded for 8-9 days following S/C infection and for 6-8 days yolk sac infection in 4-day-old ducklings. **Aguire *et al.*, (1992)** also stated that A.*hydrophila.* shedded from wild black – Bellied Whisting ducks 15% of other organisms the reisolation of A.*hydrophila.* from heart blood and liver were 100% in all groups but from kidneys were varied, 100% in I/P and I/M group and 50% in S/C group. From lungs were 66.6% in I/P and I/M groups and 50% in S/C group. The reisolation of A.*hydrophila* from brain was the lowest one as it was 33.3% in I/P & I/M groups and 0% in S/C group. **Shane and Gifford, (1985)** also reisolated A.*hydrophila* from the liver , lung , heart blood, yolk sac and brain of dead ducklings inoculated via the yolk sac .The application of in vitro antibiotic sensitivity test on A.*hydrophila* indicated that it was highly sensitive to kanamycin , streptomycin tetracycline , lencospectin , neomycin and oxytetracyclin. These results are in harmony with **Jindal *et al.*, (1993); Ghith, (1995) and Mustafa, (1996).**

From the previously mentioned results it have been concluded that natural infection of A.*hydrophila* can be contracted from fish to ducks and consequently from ducks to other ducks by contact infection. A fact that may

be considered in tracing the epidimicity of A.*hydrophila* in ducks rearing programe for efficient controlling and preventing of infection.

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Table (1): Moralities associated with experimental *A.hydrophila* infection in different groups of ducks.

*Group	Route of infection	Pattern of deaths per days (days post-infection)									Cumulative total	Mortality %
		1	2	3	4	5	6	7	14	21		
G1	I/P	0	2	1	1	0	0	0	0	0	4	40
G2	Contact to I/P	0	0	0	0	0	0	0	0	0	0	0
G3	I/M	0	2	1		0	0	0	0	0	3	30
G4	Contact to I/M	0	0	0	0	0	0	0	0	0	0	0
G5	S/C	0	1	1	-	0	0	0	0	0	2	20
G6	Contact to S/C	0	0	0	0	0	0	0	0	0	0	0
G7	Orally	0	0	0	0	0	0	0	0	0	0	0
G8	Contact to orally	0	0	0	0	0	0	0	0	0	0	
G9	Non infected control	0	0	0	0	0	0	0	0	0	0	00.0

* 10 ducklings /group

Table (2): Reisolation of *A.hydrophila*. from different organs of dead and scarified control ducks.

Groups Route infection		*A.hydrophila* reisolations from organs									
		Heart blood		Liver		Kidneys		Lung		Brain	
		n.	%	n.	%	n.	%	n.	%	n.	%
G1	I/P	3/3	100	3/3	100	3/3	100	2/3	66.6	1/3	33.3
G3	I/M	3/3	100	3/3	100	3/3	100	2/3	66.6	1/3	33.3
G5	S/C	2/2	100	2/2	100	1/2	50	1/2	50	0/2	0
G9	Control	0/3	0	0/3	0	0/3	0	0/3	0	0/3	0%

Table (3): Showing shedding of *A.hydrophila*. in infected and contact non infected ducks.

Groups	Route of infection	Positive samples Days									Percentage %
		0	1	2	3	4	7	10	14	21	
G1	I/P	(A)0/5	4/5	5/5	5/5	5/5	5/5	4/5	3/5	0/5	77.5
G2	Contact to I/P	0/5	0/5	0/5	3/5	4/5	5/5	4/5	0/5	0/5	40
G3	I/M	0/5	0/5	3/5	4/5	5/5	5/5	4/5	3/5	0/5	60
G4	Contact to I/M	0/5	0/5	0/5	3/5	4/5	5/5	3/5	0/5	0/5	37.5
G5	S/C	0/5	0/5	3/5	4/5	5/5	4/5	0/5	0/5	0/5	40
G6	Contact to S/C	0/5	0/5	0/5	3/5	4/5	3/5	3/5	0/5	0/5	32.5
G7	Orally	0/5	0/5	4/5	4/5	3/5	3/5	0/5	0/5	0/5	35
G8	Contact of orally	0/5	0/5	0/5	2/5	3/5	0/5	0/5	0/5	0/5	12.5
G9	-ve Control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0

(A): No of + ve samples / No of examined samples

Table (4): Results of the effect of different antibiotics on the *A. hydrophila* in vitro.

Antibiotic discs	Unit	Standard zone diameter	Recorded inhibitory zone diameter	Sensitivity
Kanamycin	30ug	13-18	25	+ + +
Streptomycin	10 ug	11-15	20	+ + +
Tetracycline	30 ug		22	+ + +
Ampicilin	30 ug	11-15	13	+ +
Spectenomycin + lincomycin	30 ug		26	+ + +
Neomycin sulphate	30 ug		25	+ + +
Erythromycin	30 ug	13-18	10	+
Colistin sulphate	10 ug		0	–
Chloromphenicol	30 ug	12-18	16	+ +
Amoxycillin	30 ug		17	+ +
Gentamycin	10 ug	12-13	0	–
Nalidixic acid	30 ug	13-19	15	+ +
Nitrofurantion	30 ug	14-17	10	+
Oxytetracycline	30 ug	14-19	20	+ + +

N.B.: The sensitivity test was carried out according to **Lennette *et al.*, (1980) and Collins *et al.*, (1989).**

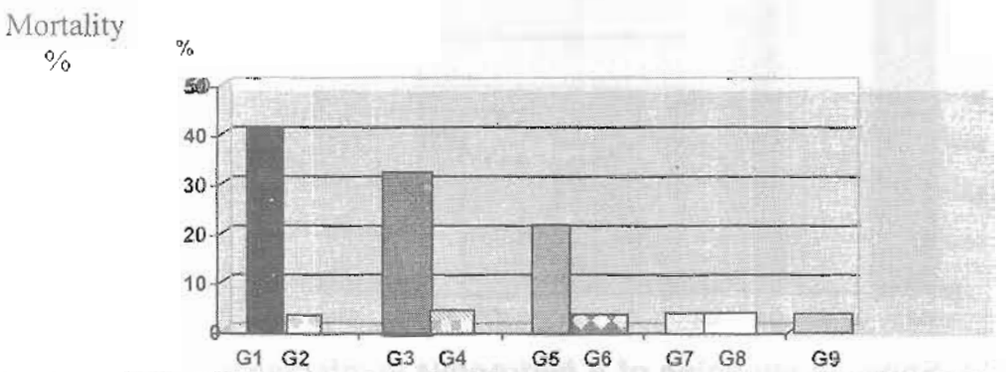

Fig. (1): Mortalities associated with experimental *A. hydrophila* infection in different groups of ducks.

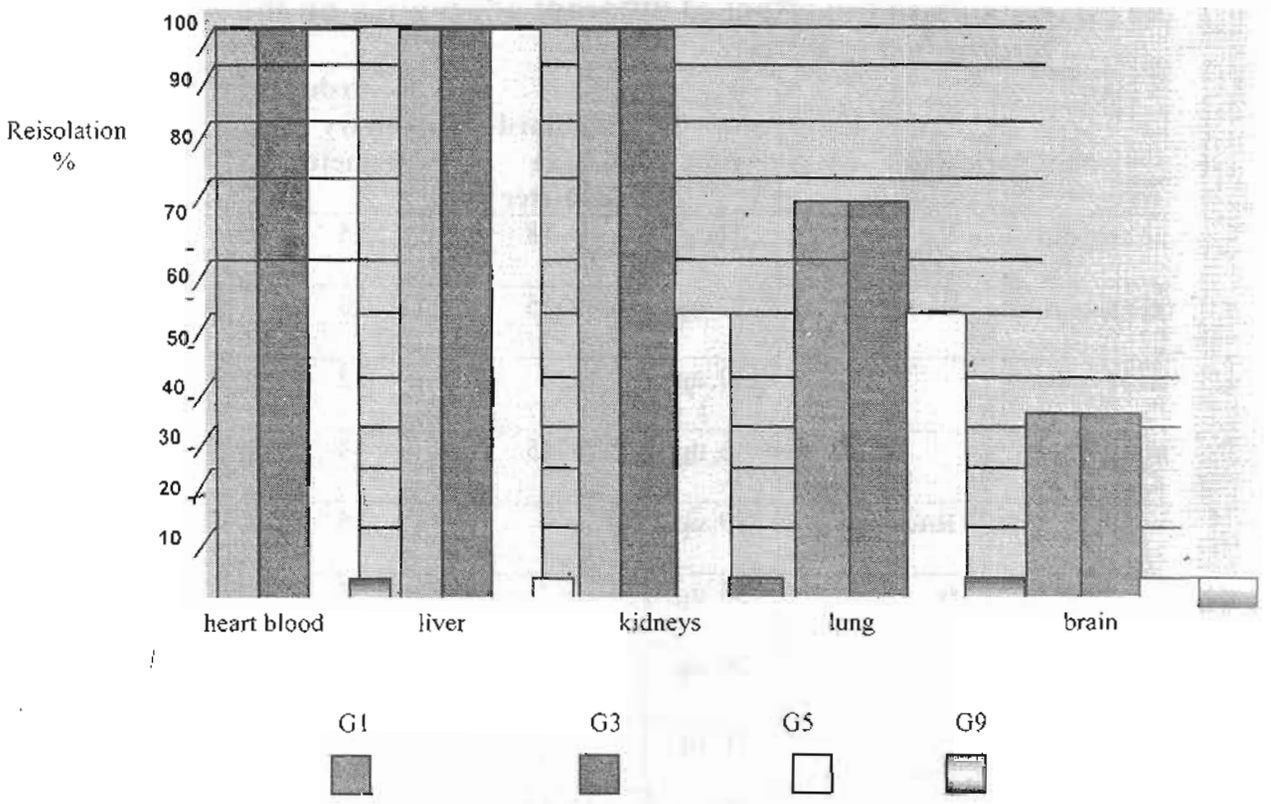

Fig (2): Reisolation of A. hydrophila from different organs of dead and scarified control ducks.

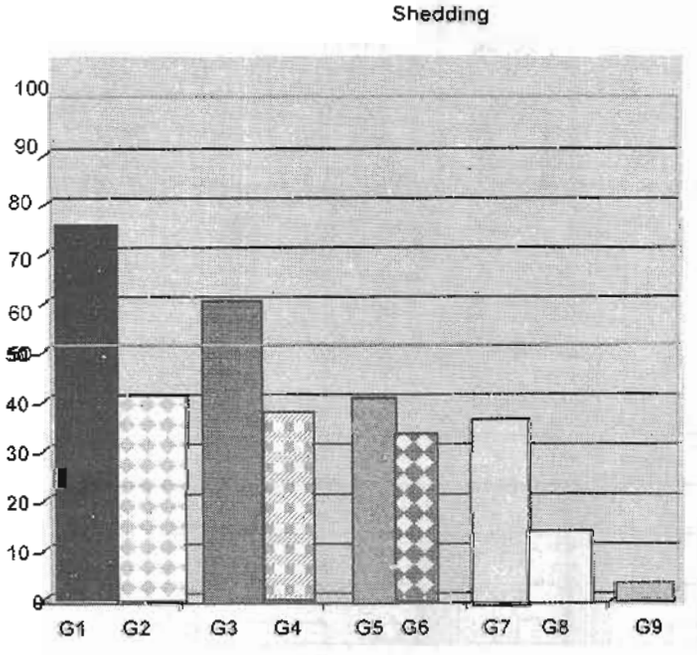

Fig. (3): Showing shedding of *A.hydrophila* in infected and contact non infected ducks.

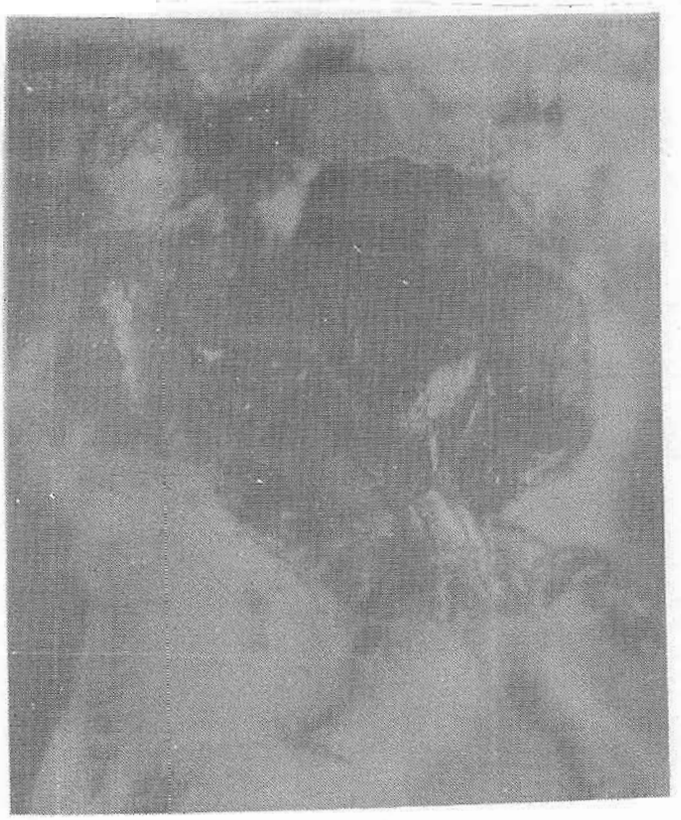

Photo (1)

Photo (2)

Photos (1-2): Showing digestion of internal organs especially liver which have hemorrhages

Photo (3)

Photo (4)

Photos (3-4): Showing digestion of internal organs especially liver which have hemorrhages

Photo (5)

Photo (6)

Photos (5-6): Showing inflammation of kidneys with distention of ureters with urates

الملـــخص العربي

مدى وبائية عدوى ميكروب الايروموناس هيدروفيلا في البط منقولة من السمك

إلهام فؤاد الخشاب وهايدي شوقي أبو اليزيد

لدراسة إحدى العلاقات المرضيـة التـي قـد توجـد فـي مـزارع الأسـماك المشـتركة المحملة بالبط . تم عدوى بط في عمر ٢١ يوم بميكروب الايروموناس هيدروفيلا (أ.هـ) تم عزله سابقا من السمك بطـرق عـدوى مختلفـة : فـي الغشـاء البريتوني - فـي العضـل - تحت الجلد ، بالإضافة إلى العدوى عن طريق الفم ، وكانت هناك عدة مجموعات ملاصقة لكل مجموعة من مجموعات العدوى المختلفة (في نفس المكان) .

كانت الأعراض و الصفة التشريحية ، و نسبة النفوق تختلف حسب طريقة العدوى . تراوحـت نسـبة النفـوق مـن ٤٠% (فـي حالـة العـدوى فـي الغشـاء الـبريتوني) و صفر% في (حالة العدوى عن طريق الفم و أيضـا المجموعـات الملاصقـة) . تـم إعـادة عزل الميكروب من الأعضاء الداخلية المختلفة لكن بنسب متفاوتة .

كما تم دراسة إفراز الميكروب في زرق الطيور و قد لوحظ أن كل الطيـور التـي تم أحداث العدوى بها و كذا الطيور الملاصقـة لـها قـد أفـرزت الميكـروب ولكـن لفـترات مختلفة .

بإجراء اختبار الحساسية لميكروب و جد انه حساس لعدة مضادات حيوية منها الكاناميسين و التتراسيكلين .