BACTERIOLOGICAL AND HISTOPATHOLOGICAL STUDIES ON SOME BACTERIAL PATHOGENS CAUSING DISEASES IN CULTURED *MUGIL CAPITO* FISH IN ISMAILIA GOVERNORATE.

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Abstract

Total number of 52 clinically diseased Mugil capito fish were collected from private fish farm (El-Kantara west) in Ismailia Governorate and subjected to clinical, postmortem, bacteriological and histopathological studies. The common clinical signs were darkness of skin, increased in mucous secretion, hemorrhages and congestion in gill cover, anal opening, mouth and base of fins. The postmortem findings were pale anemic liver in some cases and in other cases the livers were hemorrhagic and congested. Kidneys and spleen were enlarged and congested. Intestine was inflamed, hemorrhagic and free from any food particles. The predominant isolated strains were Aeromonas hydrophila (44.2%), Pseudomonas fluorescence (18.3%) and Vibrio alginolyticus (37.5 %). Results of antibiogram revealed that, ciprofloxacin and nalidixic acid were effective against A.hydrophila while ciprofloxacin and rifampicine were effective against Ps.fluorescens but V.alginolyticus isolates were sensitive to ciprofloxacin. The Pathogenicity of isolated strains were done by I/P injection of Mugil capito and the obtained results showed that A.hydrophila was pathogenic at $2X10^6$ cfu/ ml causing 90% mortality. Ps.fluorescens was pathogenic at $3X10^6$ cfu/ ml causing 80% mortality. While V.alginolyticus was pathogenic at 10^7 cfu/ ml causing 100% mortality.

Histopathological studies on experimentally infected Mugil capito with A.hydrophila, Ps.fluorescens and V.alginolyticus showed degenerative changes in the internal organs especially liver, kidney and spleen.

Introduction

Aquaculture has an important role in the development and meeting the increase demand for aquatic animal production *(Haylor and Bland, 2001)*. Aquaculture industry gradually developed in the world as well as in Egypt. The health keeping of fish depends on the relationship between fish; environment and pathogens. Fish is an essential source of high nutritive value with good digestibility and

cheap source of animal protein. Fish is susceptible to wide variety of bacterial pathogens. Primary and secondary bacterial diseases account 80% of fish mortalities *(Snieszko, 1976)*. Mugil species culture is one of an important aquaculture activity in Egypt and other countries, the major factor which would hamper its succe-ssful development and sustainability would be diseases *(Austin and Austin, 1993)*. Bacterial diseases are the most common diseases in intensive fish rearing facilities *(Kusuda and Salati, 1999)*. Outbreaks of diseases attributed to bacterial pathogens are devasting to both culture and wild fish populations *(Austin and Austin, 1993). (Wafeek et al 2007)* isolated V.alginolyticus from Grey Mullet fish (Mugil cephalous) collected from Sharme El - Sheikh with high percent of 39%. *(Ahmed, 2004)* isolated Ps. fluorescens from naturally infected Mugil species with percentage 21.33%.The clinical signs of infected fish make them unmarketable *(Samaha et al, 2004)*. The present study was planned to investigate the most common bacterial pathogens affecting Mugil capito, studying antibiogram, Pathogenicity test and histopathological alterations in experimentally infected fish with isolated bacteria.

Material and methods

Fish;

A total number of 52 naturally diseased Mugil capito were collected from private brackish water fish farm(El kantara west) in Ismailia Governorate and transferred alive in oxygenated plastic containers to the Laboratory of Microbiology Department, Faculty of Veterinary Medicine, Suez Canal University and subjected to full clinical and postmortem examination as described by *Schaperclaus et al, (1992)*.

Bacteriological examination;

A total number of 260 Samples were aseptically taken form mouth, gills, liver, kidneys and spleen (52 samples from each organ) and cultivated on Tryptic Soy agar (Adwic), TCBS agar and R.S media (Oxoid) and incubated at 25°C for 24 hrs. Purified isolates were identified by standard biochemical tests according to *(Bergey, et al, 1984), (Austin and Austin, 1987) and (Schaperclaus et al, 1992)*.

Antibiogram;

Sensitivity of isolated bacterial pathogens Aeromonas hydrophila, Pseudomonas fluorescens and Vibrio alginolyticus to different antibiotics was estimated according to *(Baur et al; 1966)*.

Pathogenicity test;

A total number of 40 Mugil capito fish were collected alive and apparently healthy from private hatcheries in Manzala city with an average body weight (20±5 g.) and were tested for susceptibility to experimental infection with A.hydrophila, Ps.fluorescens and V.alginolyticus isolated from naturally infected Mugil capito. Fish were maintained in glass aquaria supplied with well aerated dechlorinated tap water to be acclimated. All experimental fish were fed with commercial ration at rate of 5%

body weight per day. Four groups were classified (10 fish each).The first group were inoculated I/P with 0.5 ml of saline containing A.hydrophila with ($2x10^6$ cfu /ml) *Luky, (1977).* The 2nd group were inoculated I/P with 0.5 ml of saline containing Ps.fluorescens with ($3x10^6$ cfu/ml) *Luky, (1977).* The 3rd group were inoculated I/P with 1.0 ml of saline containing V.alginolyticus with (10^7 cfu/ml) *Mustafa, et al (1990).* The 4th group was left as control and injected I/P with 0.5 ml sterile saline. All experimentally injected fish were observed daily for 3-5 weeks to record any clinical signs and mortalities. Postmortem examination was done on freshly dead fish to record gross lesions .Bacteriological re-isolation of A.hydrophila, Ps. fluorescens and V.alginolyticus were attempted from freshly dead and scarified fish.

Histopathological examination

Specimens for histopathological techniques were freshly taken from gills, liver, kidney and spleen of experimentally infected fish. Histopathological techniques were carried out according to *Roberts, (2001).*

Results and Discussion

The common clinical signs of naturally bacterial infected fish were darkness of skin, increase in mucous secretion, hemorrhages and congestion of gill cover; anal opening, mouth and base of fins (Photo 1, 2). The results of postmortem examination showed pale anemic liver (Photo 3) in some cases and liver was congested and hemorrhagic in other cases. Kidneys and spleen were hemorrhagic and congested **(Photo4),** intestine was inflamed, hemorrhagic and free from any food particles; excessive mucous secretion and hemorrhages were due to toxin and proteolytic enzymes of pathogenic strains. The results of bacteriological examination of naturally infected Mugil capito showed that the most common isolated bacteria were A.hydrophila, V.alginolyticus and Ps.fluorescens as 46 isolates (44.2%) 39 isolates (37.5%) and 19 isolates (18.3%) respectively as shown in (Table 1). The high prevalence of A.hydrophila could be attributed to its presence as a part of intestinal flora in healthy fresh and marine water fish. Regarding to distribution of isolated strains in different organs (Table 2) shows the prevalence of different pathogens in mouth, gills. Liver, kidney and spleen were 38.46, 34.6, 11.53, 9.6 and 5.8% respectively. In the present study, A.hydrophila was isolated from Mugil capito with percentage (44.2%). These results nearly agree with *Austin and Austin, (1987)* where Motile Aeromonas septicemia (MAS) is considered as one of the common bacterial causes of fish mortalities, and agree with *Ahmed, (2004)* who recorded that, the common bacterial pathogen isolated from naturally infected Mugil cephalus was A.hydrophila with percentage 46.67%. The distribution of A.hydrophila in different organs was 39.13, 43.47, 6.52, 6.52 and 4.34% in mouth, gills, liver, kidney and spleen

respectively.

In the present study Ps.fluorescens was isolated from Mugil capito with percentage (18.3%). These results agree with *Schaperclaus et al., (1992),* where Pseudomonas species has been considered as secondary invader of damaged fish tissue as well as a primary poor and weak pathogen. *Ahmed, (2004)* who isolated Ps.fluorescens from naturally infected Mugil species with percentage 21.33 %, the distribution of Ps.fluorescens in different organs was 36.84, 31.57, 15.78, 10.52 and 5.26% in mouth, gills, liver, kidney and spleen respectively that agreed with the present study. In the present study V.alginolyticus was isolated from Mugil capito with percentage 37.5%. These results agree with *Wafeek et al (2007)* who isolated V.alginolyticus from Grey mullet fish (Mugil cephalus) collected from Sharm El-Sheikh with high percent of 39%. The distribution of V.alginolyticus in different organs was 38.5, 25.64, 15.38, 12.82 and 7.69% in mouth, gills, liver, kidney and spleen respectively. Antibiogram test was carried out on isolated bacterial strains as shown in Table (3) A.hydrophila strains were sensitive to ciprofloxacin and nalidixic acid, intermediate to rifampicin while resistant to colistin sulfate, erythromycin, amoxicillin, amikacin and lincomycin (Photo7). Ps.fluorescens strains were sensitive to ciprofloxacin and Rifampicine, intermediate to nalidixic acid, while resistant to colistin sulfate, amikacin, amoxicillin, lincomycin and erythromycin (Photo 8). V.alginolyticus strains were sensitive to ciprofloxacin, intermediate to Rifampicine and nalidixic acid, resistant to amoxicillin, colistin sulfate, Lincomycin, amikacin and erythromycin (Photo 9).

Results of pathogencity test Table (4) showed that The clinical signs of the disease were seen after 24 hrs post I/P injection with A.hydrophila which included loose scales, inflammatory changes at the site of inoculation (Photo 5) hemorrhages allover different parts of the body (Photo 6) and presence of peticheal hemorrhages and congestion in liver, kidneys and spleen .Reisolation of A.hydrophila was obtained on RS media from all freshly dead fish, mortality rate was 90 %. Intraprotinally injected fish with Ps.fluorescens showed loss of balance, skin discoloration with scattered hemorrhages all over the body surface. Re-isolation of Ps.fluorescens was obtained from all freshly dead fish, mortality rat was 80%. Intraprotinally injected fish with V. alginolyticus showed dark discoloration of skin, ulcers and hemorrhages all over the body surface. Liver, kidneys and spleen were enlarged and congested. Re-isolation of V.alginolyticus was obtained from all freshly dead fish. Mortality rate was 100%.

Concerning histopathological studies the liver showed vacuolar degeneration, congestion of central vein, hyperplasia of epithelial lining the bile duct with budding of newly formed bileductules, such hepatic lesions were indicative of septicemia as the liver was damaged by pathogenic

bacteria and its toxin. The kidney in the present work showed hyaline droplet degeneration, the variation in lesions could be due to the difference in bacterial virulence and degree of individual resistance of fish. The hyaline droplet degeneration suggests the existence of glomerular disease which can result in protein leakage into the filtrate and decreased osmotic pressure with its consequences. The hyaline droplet degeneration is recognized histologically as homogenous esinophilic intracytoplasmic droplet as a result of reabsorption of albumin from renal filtrate in case of protein urea *(Ferguson 1989)*.The spleen in this study showed increase in number of melanomacophage cells (Photo 10), the activation of melanomacophage center in the spleen is assumed to be a result of stimulation of this organ by mild action of bacterial toxin. The gills in this study showed hyperplasia of secondary lamellae, congestion of gill arch and telangictasia Photo (11) and (12). *Kudo and Kimura, (1985) and Wakabayashi and Iwado, (1985)*, suggested that the bacteria produce an extracellular hyperplasia inducing factor which can produce typical lesions as epithelial hyperplasia associated with lamellar fusion. While the telangictasis of secondary lamellae was produced as a response to branchial injury in which there is break down of vascular integrity due to rupture of pillar cells and pooling of blood *(Ferguson 1989)*.

Table (1) Prevalence of bacterial isolates from examined naturally infected Mugil capito.

No. of samples	No.of isolates	*A.hydrophila*	*Ps.fluorescens*	*V.alginolyticus*
260	104	46	19	39
%	40	44.2	18.3	37.5

Table (2) distribution of isolated strains in examined tissues and organs.

Organs	*A.hydrophila*		*Ps.fluorescens*		*V.alginolyticus*		Total	
	NO	%	NO	%	NO	%	No	%
mouth	18	39.13	7	36.84	15	.46٣٨	40	38.46
Gills	20	43.47	6	31.57	10	25.64	36	34.61
Liver	3	6.52	3	15.78	٦	15.38	12	11.53
Kidney	3	6.52	2	.52١٠	٥	٢.8١٢	10	9.61
Spleen	2	4.34	1	.26٥	٣	7.69	6	5.76
Total	46	44.2	19	.318	٣٩	.5٣٧	104	40

Table (3) Antibiotic sensitivity of isolated bacterial strains

Antibiotic disc	Symbol	Concentration (Mcg)	*Sensitivity reactions*		
			A.hydrophila	*Ps.fluorescens*	*V.alginolyticus*
Ciprofloxacin	Cip_5	5	S	S	S
Colistin sulfate	Cl_{10}	10	R	R	R
Rifampicine	RD_{30}	30	I	S	I
Nalidixic acid	Na_{30}	30	S	I	I
Amoxicillin	Amx_{25}	25	R	R	R
Erythromycin	E_{15}	15	R	R	R
Amikacin	AN_{30}	30	R	R	R
Lincomycin	L_2	2	R	R	R

R=Resistant **I**= Intermediate **S** =Sensitive

Table (4) Mortality rate of experimentally infected fish with *A.hydrophila, PS. Fluoresces and V.alginolyticus.*

Group	Dose per fish	Fish No	Dead fish during 7 day after injection							No.of dead fish	MR %
			1	2	3	4	5	6	7		
1	*A.hydrophilla* 0.5 ml $2x10^6$	10	1	3	2	3	-	-	-	9	90
2	*Ps.fluorescens* 0.5 ml $3x10^6$	10	2	2	1	2	1	-	-	8	80
3	*V.alginolyticus* 1 ml $x10^7$	10	2	1	3	4	-	-	-	10	100
4	Control group 0.5 ml sterile saline	10	-	-	-	-	-	-	-	-	0

MR%=Mortality Rate

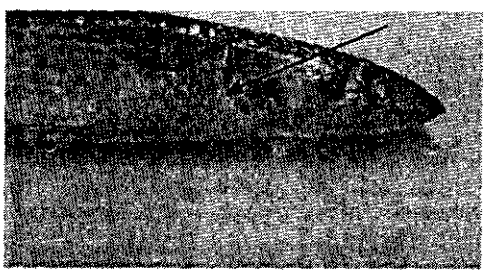

Photo (1) naturally infected *Mugil capito* showed hemorrhages on gill cover.

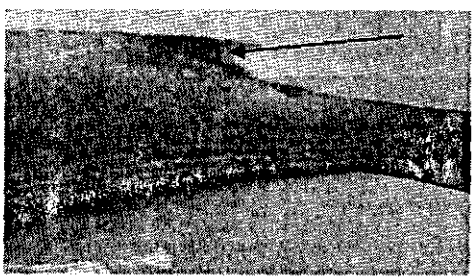

Photo (2) naturally infected *Mugil capito* showed peticheal hemorrhages around anal fin.

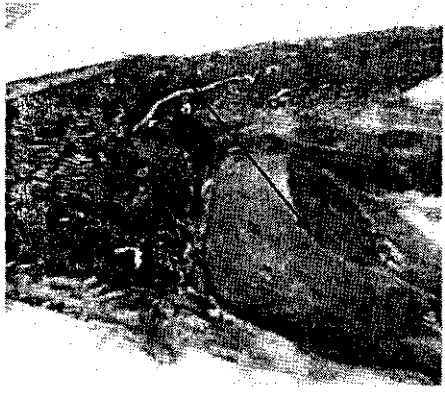

Photo (3) naturally infected *Mugil capito* showed pale anemic liver.

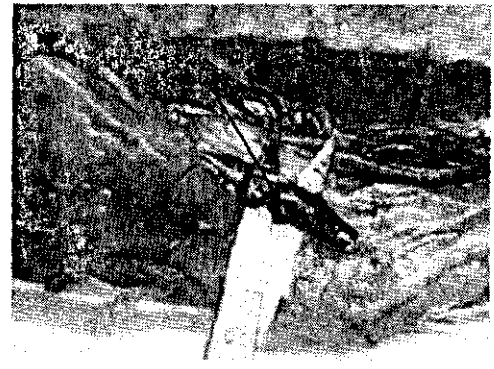

Photo (4) naturally infected *Mugil capito* showed enlarged congested spleen.

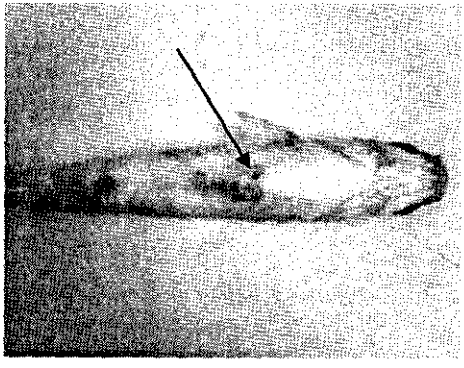

Photo (5) experimentally infected *Mugil capito* showed hemorrhages at site of bacterial inoculation

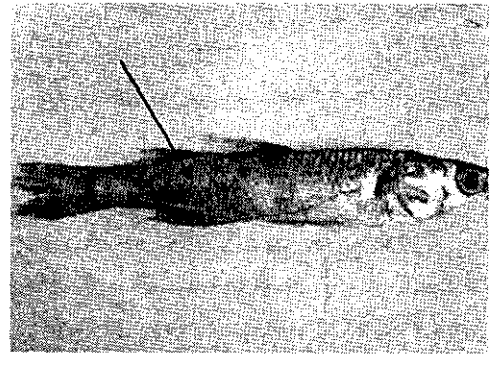

Photo (6) experimentally infected *Mugil capito* showed hemorrhages allover pats of external body.

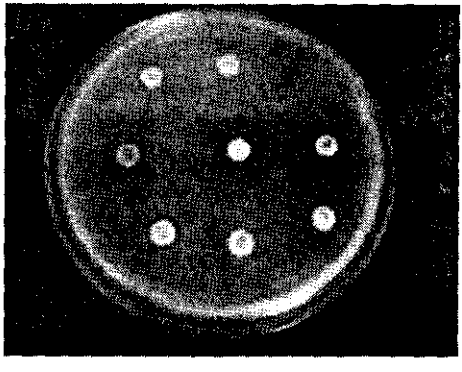

Photo (7) Antibiogram of *A.hydrophila.*

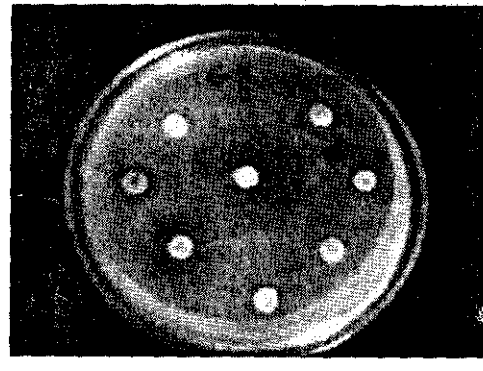

Photo (8) Anti'biogram of *Ps.fluorescens.*

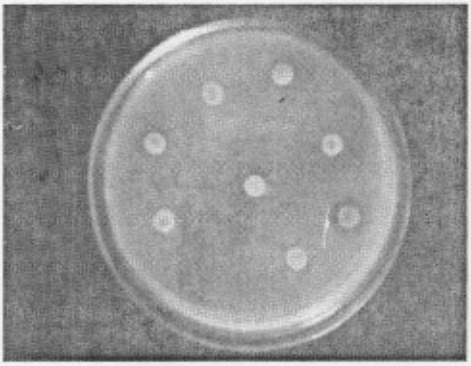

Photo (9) Antibiogram of *V.alginolyticus*

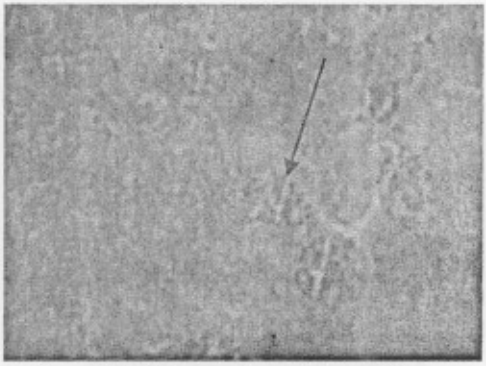

Photo (10) Spleen of experimentally infected *Mugil capito* showed increase in number of melanomacrophage cells.

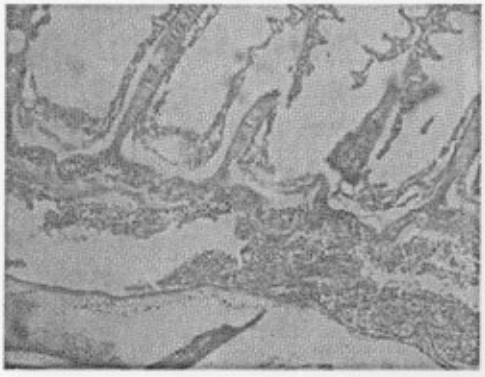

Photo (11) Gills of experimentally infected *Mugil capito* showed hemorrhages in gill arch.

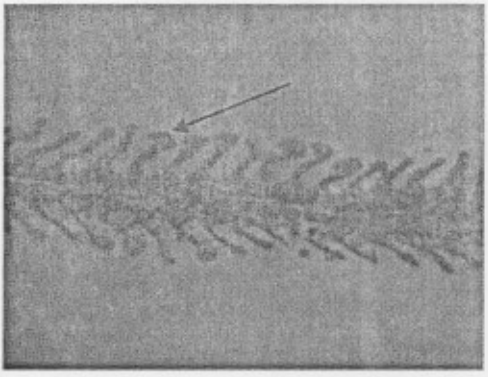

Photo (12) Gills of experimentally infected *Mugil capito* showe telangactasis

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الملخص العربى

دراسات بكتريولوجية وهستوباثولوجية على بعض البكتيريا الممرضة فى اسماك الطوبارالمستزرعة فى محافظة الاسماعليه.

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تم اجراء هذة الدراسة على عدد ٥٢ سمكة من اسماك الطوبار المرباة فى مزرعة خاصة بالقنطرة غرب محافظة الاسماعلية وتمثلت العلامات المرضية فى وجود انزفة على الجلد والغطاء الخيشومى والزعانف الفتحة الشرجية والخياشيم. وتمثلت الصفة التشريحية لهذة الاسماك فى وجود تضخم واحتقان فى الطحال و الكلى وكان الكبد ما بين البنى الداكن الى الاصفر الباهت اللون. تم اخذ ٢٦٠ عينة من (الفم – الخياشيم- الكبد- الطحال- الكلى) بمعدل ٥٢ عينة من كل عضو وتم العزل على البينات البكتيرية المختلفة لعزل المسببات البكتيرية وقد تبين من الفحص البكتيريولوجى للأسماك المصابة طبيعيا عزل ١٠٤ عتره بكتيرية بنسبة إصابة ٤٠% وقد تم دراسة الخواص البيوكميائية للعترات المعزولة وذلك لتصنيفها وقد وجد أنها تنتمي إلى٣ فصائل بكتيرية وكانت نتائج العترات كالتالي:
- البكتيريا السائدة كانت الايروموناس هيدروفيلا ٤٦ عترة بكتيرية بنسبة إصابة ٤٤,٢% يليها الفيبروألجينولتيكس ٣٩ عترة بكتيرية بنسبة إصابة ٣٧,٥ % بالإضافة إلى السودموناس فلوروسنس ١٩ عترة بكتيرية بنسبة إصابة ١٨,٣%
- اثبت اختبار الحساسية المعملي أن السبروفلوكساسين و النالدكسيك أسيد هما المضادان الحيويان الأكثر فاعلية بالنسبة لميكروب الايروموناس هيدروفيلا بينما السبروفلوكساسين والريفامبيسين بالنسبة لميكروب السودوموناس فلوروسنس والسبروفلوكساسين في حالة الفيبروألجينولتيكس.
أوضحت العلامات والصفات التشريحية في الأسماك المصابة صناعيا نفس العلامات في الأسماك المصابة طبيعيا- وكانت نسبة النفوق نتيجة الحقن البروتونى لأسماك الطوبار بميكروب الايروموناس هيدروفيلا ٩٠% أما المجموعة المحقونة بميكروب الفيبروألجينولتيكس. فكانت نسبة النفوق ١٠٠% أما المجموعة المحقونة بميكروب السودوموناس فلوروسنس فكانت نسبة النفوق ٨٠% .
أوضحت الدراسة الهستوباثولوجية لأسماك الطوبار المحقونة صناعيا وجود تغيرات مرضية في الأعضاء الداخلية والتى كان من اهمها وجود تنكرز موضعى واحتقان بالاوعية الدموية للكبد وارتشاح اوديمى ، وفى الكلى وجد تنكرز بالقنوات الكلوية و احتقان وارتشاح اودمى ، ووجد بالطحال تكاثر لمراكز الميلانومكروفاج ، وفى الخياشيم وجد احتقان وارتشاح اودمى فى القوس الخيشومى مع تمدد فى الخلايا المبطنة للورقة الخيشومية.