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HISTOPATHOLOGICAL AND IMMUNOLOGICAL STUDIES ON AEROMONAS HYDROPHILA INFECTION IN COMMON CARP

By

SAMIA A. AHMED*, M.S. MARZOUK*, ROUFAYDAH M.A. MEGID**, M. MOUSTAFA* and M.GADO***

* Dept. Int. Med., Infec. Dis. and Fish., Fac. Vet. Med.Cairo Univ.
** Dept .Microb., Fac. Pharm., Cairo Univ.
***Dept. of Fish Dis., Fac. Vet. Med. Kafr El-Sheikh, Tanta University.

SUMMARY

The histopathological changes induced by *Aeromonas hydrophila* (A.hydrophila) organism among common carp (Cyprinus carpio L.) at Kafr El-Sheikh Governorate was investigated. The immune response of common carp inoculated with formalized whole culture and formol toxoid vaccines of *Aeromonas hydrophila* was also studied.

INTRODUCTION

Fish aqua-culture projects are becoming increasingly important in supplementing cheap animal protein of good quality all over the world. The expansion in aqua-culture productivity is interrupted in some instance with some disease problems among which Motile Aeromonas Septicaemia (MAS) is a more drastic one (Kaper et al., 1981). The presence of a wide variety of *A.hydrophila* strains with different antigenic properties represent a major problem in controlling this organism and usually leads to high economical losses (Lallier et al.,1980). The present study was planned to investigate the pathological effect of *A.hydrophila* in cultured common carp well as to apply immunological studies aiming for control of such infection.

MATERIAL AND METHODS

1. Fish.

A total number of 310 clinically normal carp (average body weight of 90g ± 5/ fish) was collected from fish farm at Kafr El-Sheikh Governorate. The fishs were adapted to a water temperature of 20 ± 2°C in the laboratory of fish disease, Faculty of Vet. Medicine, Cairo University to be used in the experimental infection.

2. A. hydrophila Bacteria;

A well identified, virulent *A.hydrophila* bacterial isolate was kindly supplied by laboratory of fish diseases and management, Fac. of Vet. Med. Cairo University. The organism was propagated on trypticase soy agar (TSA) and trypticase soy broth (TSB) to be used for experimental inoculation of common carp.

3.Histopathological examination of experimentaly inoculated Fish:

A total number of 10 clinically normal common carp was inoculated each with 0.2 ml of 24 hour broth culture of *A.hydrophila* (1.5 x 10^9 cells / ml) intramuscularly (IM) in the back

region. The inoculated fish were observed for 10 days post inoculation.

According to the methods described by Roberts (1978) and Austin and Austin (1987); tissue specimens were taken from the affected organs namely, liver, spleen, kidney, intestine of moribund fish. Tissue specimens were fixed, sectioned (3-5μ) and stained with haematoxylin and eosin as described by Hibiya (1982) and then examined microscopically.

4. Preparation of A.hydrophila Vaccines:

a. Formalized whole vaccine:

To 24 hours broth culture of the virulent isolate of *A.hydrophila*; formalin was added to a final concentration of 2% (V/V) and left overnight at 4°C, then the density was adjusted to Macfarland standard No.5 (Equivalent to 1.5 x 10^9 cells / ml) according to Fingergold and Martin (1982).

b. Formol toxoid Vaccine:

The extracellular product (ECP) of the most virulent isolate of *A. hydrophila* was treated with formalin (0.3%, V/V) and the pH was adjusted to 7.4, then incubated for 2 weeks at 37°C. The obtained toxoid was kept in refrigerator until used (Toply and Wilsons 1990).

5. Application of A.hydrophila vaccine:

a- Formalized whole vaccine:

I- Parentral Vaccination:

Formalized whole vaccine of *A.hydrophila* was applied by parentral and immersion routes. For parentral vaccination; a ninety (90) common carp fish, weighing about 90g/ fish (±5) were divided into three groups, the first one of a thirty (30) fish was vaccinated intraperitoneally (IP) with 0.3 ml/fish of the formalized whole culture vaccine containing 1.5 x 10^9 cells/ ml (equivalent to Macferland Standard No.5) whilst the second group of thirty (30) fish was vaccinated IM with 0.5ml fish of the same formalized whole culture vaccine. The third group of thirty (30) fish was left as control.

After 28 days all groups were subdivided into two subgroups each of 15 fish and all subgroups were challenged IP and IM with 0.2 ml/fish of a broth culture of the virulent *A.hyrophila* containing 1.5 x 10^9 cells/ml.

II- Immersion Vaccination;

For immersion vaccination one hundred and twenty (120) common carp fish weighing 90 g/ fish (± 5) were used. A ninety (90) fish were vaccinated by immersion technique (Austin & Austin 1987) with a solution containing 1.5 x 10^9 /cells of the formalized whole culture vaccine. Thereafter , half a minute a group of 30 fish was transfered to a new vaccine-free aquarium, a second group of 30 fish was transfered after one minute whilst the third group of 30 fish were removed after 2 minutes of immersion. A fourth group of thirty (30) fish were left as control. After 28 days all fish groups were subdivided into 2 subgroups each of 15 fish, one group of which were challenged IP with 0.2 ml of broth culture suspension containing 1.5 x 10^9 cells/ml of virulent *A.hydrophila* isolate and the fishes of other subgroups were challenged by immersion for 2 minutes of a solution containing 1.5 x 10^9 cells/ ml of virulent *A.hydrophila*.

b. Formol toxoid Vaccine:

Formol toxoid vaccination was carried out on ninety (90) fish weighing 90 g (± 5). Thirty (30) fish was vaccinated with 0.2 ml/fish IP and the second group thirty (30) fish IM. The third group of thirty (30) fish has been

considered as control. After 28 days all groups were subdivided into two subgroups, each of 15 fish. The in all subgroups were challenged IP and IM with 0.5ml/ fish of broth culture containing 1.5 x 10^9 cells/ml. (equivalent to Macfarland standard No.5) of the virulent *A.hydrophila*. The dead and survived fish were recorded for ten days post-challenge in all the aforementioned experiments.

The vaccine potency was determined by PRP afforded by the different vaccines., and was calculated by such formula according to Ahmed (1981) as:

$$PRP = \frac{\%\text{ of mortality of vaccinated fish}}{\%\text{ of mortality of control fish}}$$

RESULTS

1. Results of Histopathological examination:

The histopathological examination of common carp inoculated IM with *A.hydrophila* revealed vascular degenerative changes and necrosis in the liver of inoculated fish. The kidneys show severe necrosis of renal epithelium with the presence of eosinophilic casts in some renal tubules. Severe congestion was also demonstrated in the splenic blood vessels which contained haemolysed blood. The intestinal wall showed massive necrosis and desquamation of the epithelial lining with inflammatory exudation. The results of the histopathological examination are shown in Figs. 1-4.

Table 1: Evaluation of formalized whole culture A.hydrophila vaccine using I/P and I/M routes of vaccination.

Route of vaccination	Challenged fish*		Dead fish**		Percentage of relative protection (PRP)***
	Route of challenge	No. of fish	No. of fish	%	
I/P route	I/P	15	2	13.3	84.6
	I/m	15	1	6.6	90.9
I/m route	I/P	15	3	20.0	76.9
	I/m	15	2	13.3	81.8
Control	I/P	15	13	86.7	-
	I/m	15	11	73.3	-

* Challenged fish; 28 days post-vaccination

** Dead fish; 10 days post-challenge.

*** $PRP = 1 - \frac{\%\text{ of mortality of vaccinated fish}}{\%\text{ of mortality of control fish}} \times 100$

Table 2: Evaluation of formalized whole culture **A.hydrophila** vaccine using immersion technique:

Route of vaccination	Challenged fish*		Dead fish**		Percentage of relative protection (PRP)***
	Route of challenge	No. of fish	No.	%	
Immersion for ½ min.	I/P	15	12	80	7.7
	Immersion	15	7	45.7	30
Immersion for 1 min.	I/P	15	9	60	30.7
	Immersion	15	5	33.3	50
Immersion for 2 min.	I/P	15	5	33.3	61.2
	Immersion	15	2	13.3	80
Control	I/P	15	13	86.7	
	Immersion	15	10	66.7	

* Challenged fish; 28 days post-vaccination

** Dead fish; 10 days post-challenge.

*** PRP = 1- $\frac{\text{\% of mortality of vaccinated fish}}{\text{\% of mortality of control fish}} \times 100$

TAble 3: Evaluation of formol toxoid vaccine of **A.hydrophila** vaccine using I/P and I/M routes of vaccination.

Route of vaccination	Challenged fish*		Dead fish**		Percentage of relative protection PRP***
	Route of challenge	No. of fish	No. of fish	%	
I/P route	I/P	15	4	26.7	69.2
	I/m	15	3	20.0	72.7
I/m route	I/P	15	6	40.0	53.8
	I/m	15	4	25.7	63.5
Control	I/P	15	13	86.7	-
	I/m	15	11	73.3	-

* Challenged fish; 28 days post-vaccination

** Dead fish; 10 days post-challenge.

*** PRP = 1- $\frac{\text{\% of mortality of vaccinated fish}}{\text{\% of mortality of control fish}} \times 100$

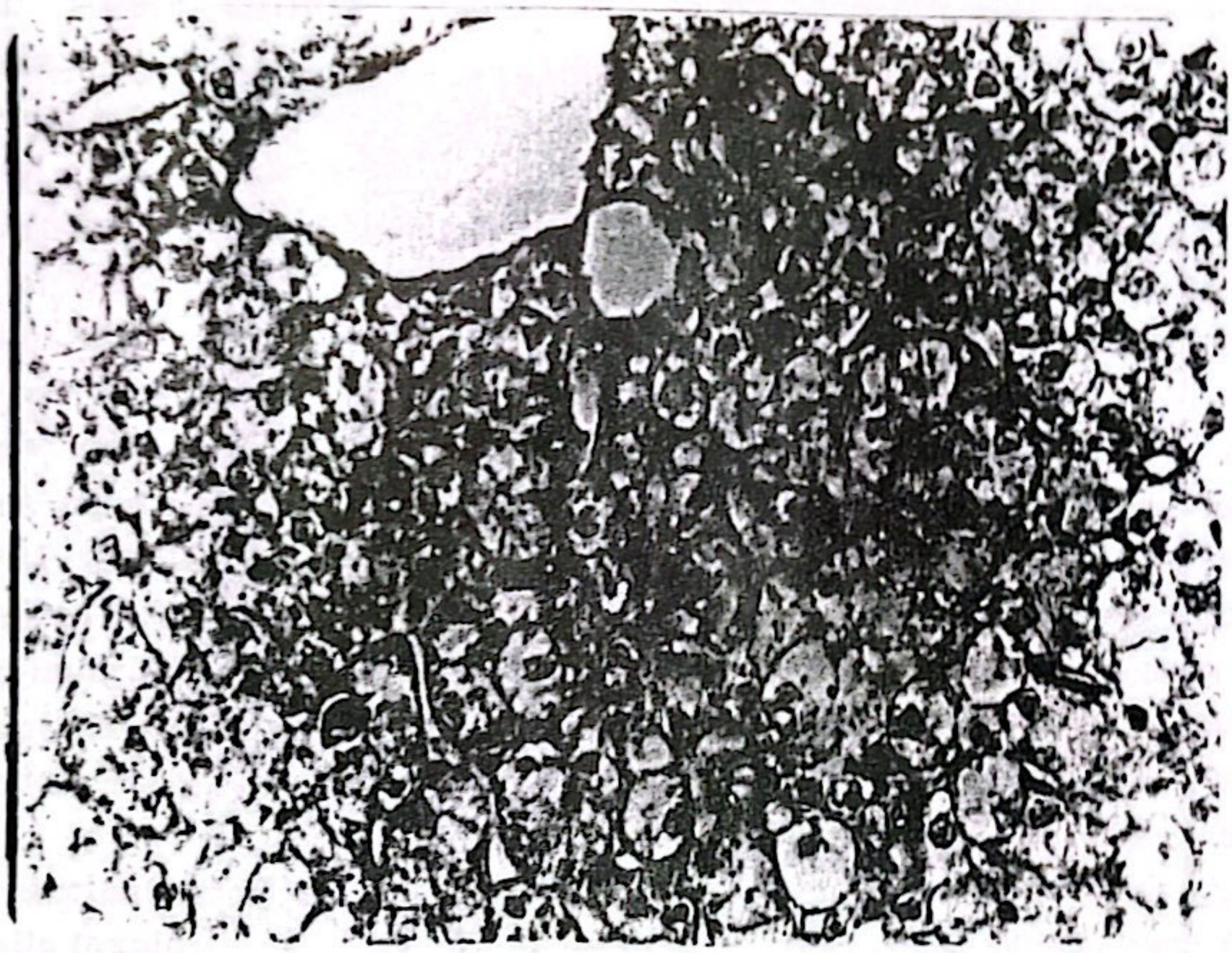

Fig. 1: Liver of common carp IM inoculated with *A. hydrophila* showing focal areas of necrobiotic changes in the hepatocytes with vascular degeneration.

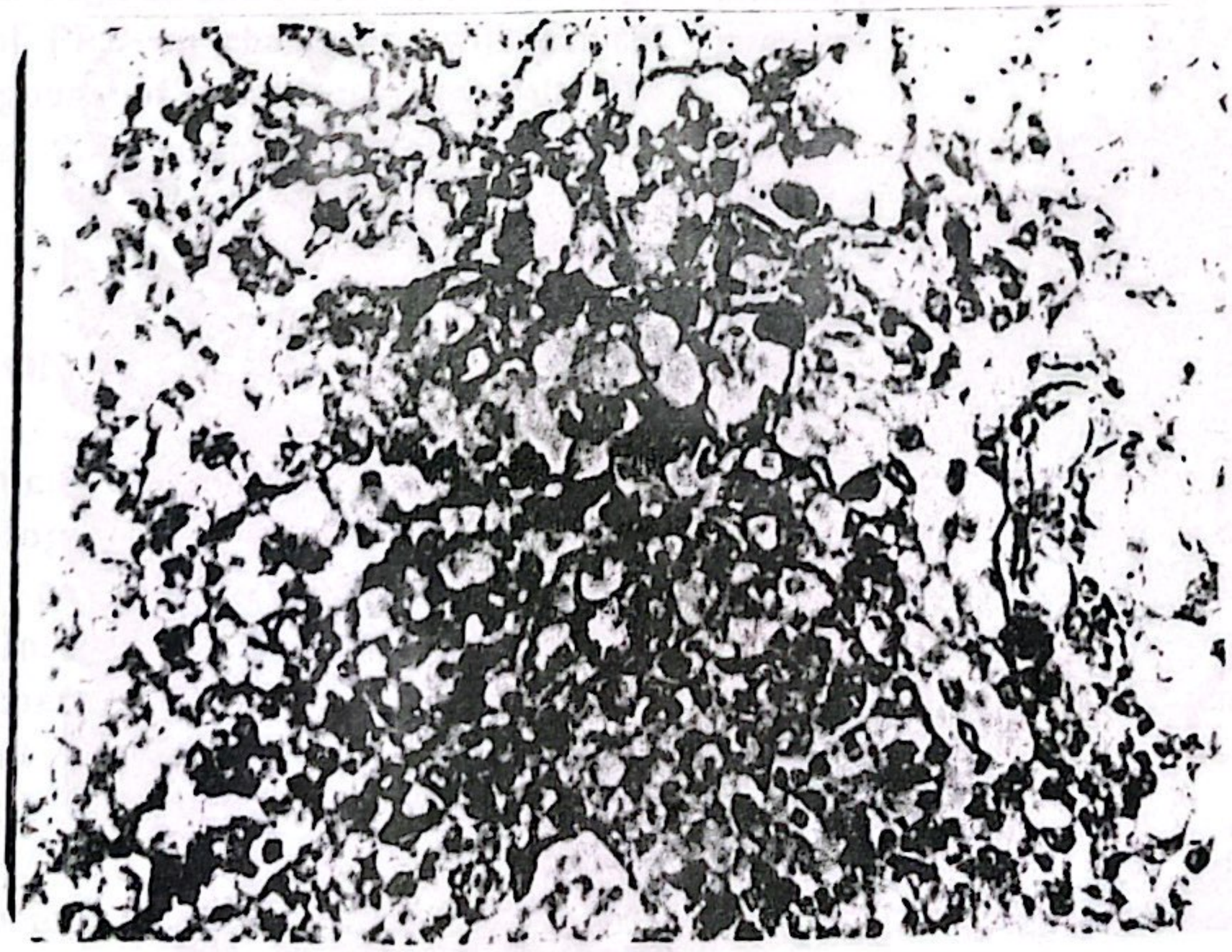

Fig. 2: Kidney of common carp IM inoculated with *A. hydrophila* showing tubular necrosis with the presence of eosinophilic casts filling some renal tubules.

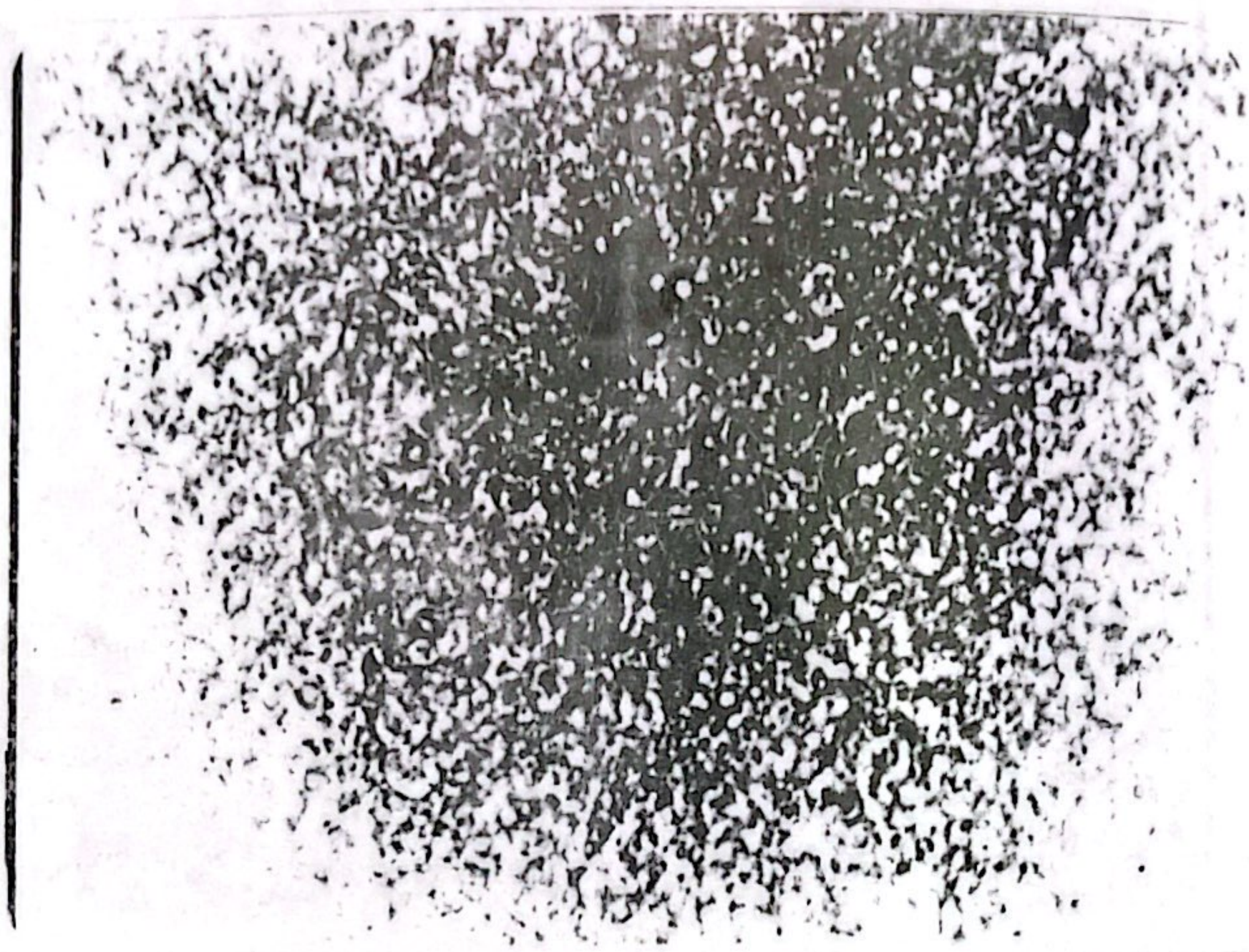

Fig. 3: Spleen of common carp IM inoculated with *A. hydrophila* showing severe congestion of the splenic blood vessels which contained haemolysed blood.

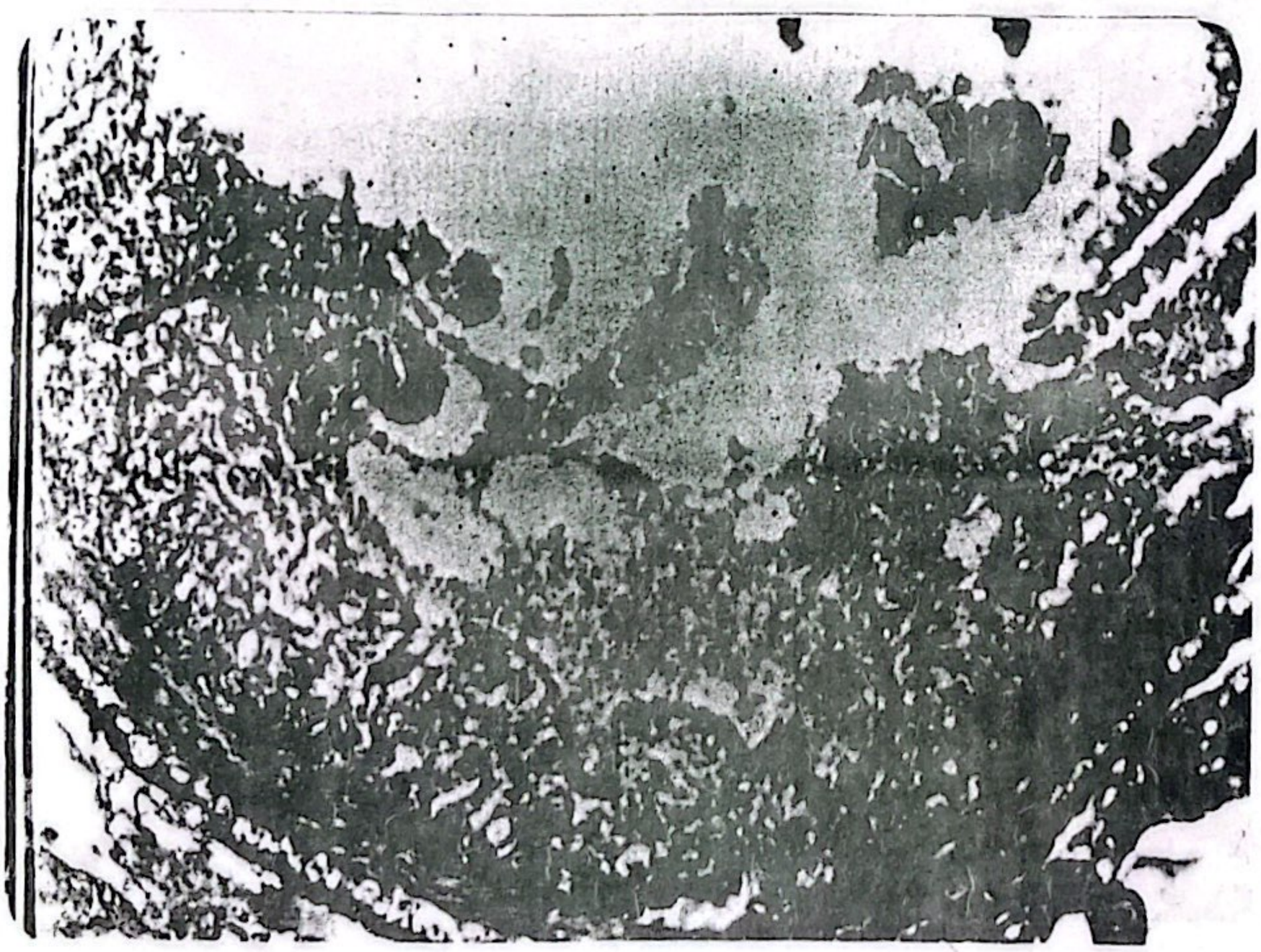

Fig. 4: Intestine of common carp IM inoculated with *A. hydrophila* showing massive necrosis of the intestinal wall with inflammatory exudation and desquamation of epithelial cells.

2- Results of vaccination of common carp with formalized whole culture vaccine of A.hydrophila.

The results of vaccination of formalized common carp with formalized whole culture vaccine prepared from the virulent *A.hydrophila* isolate by the IP and IM routes showed a percentage of relative protection (PRP); on challenge with the virulent homologous of *A.hydrophila* isolate. Also different PRP were demonstrated in common carp immunized through the immersion method after IP and immersion challenge. The result of the vaccination experiment are shown in Tables 1 and 2.

3. Results of vaccination of common carp with A.hydrophila toxoid:

The results of common carp immunization with formalized toxoid prepared from the extracellular products (ECPs) of *A.hydrophila* isolate through IP and IM routes revealed high levels of PRP on challenging with virulent homologous of *A.hydrophila* isolate. The results of this vaccination are shown in Table 3.

DISCUSSION

In the clinical diagnosis of fish disease, the histopathological investigations could be considered an important tool for accurate diagnosis. In this study the histopathological lesions in common carp experimentaly infected with virulent *A.hydrophila* were demonstrated. The liver of inoculated fish showed vascular degeneration/ with endothelial vascular damage. The haemopoietic elements of anterior kidney and spleen were depleated. The posterior kidney showed massive tubular nephrosis.

On the other hand , necrosis and sloughing, inflammatory cell infilteration were demonstrated in the intestinal mucosa and submucosa repectively.Therefore mentioned histopathological findings are more or less similar to those findings of naturally infected fish reported by many authors (Gains 1972, Bucke et al., 1975, Roberts 1978 and Ventura and Grazzle, 1988).

Regarding the immunization of common carp with formalized whole culture vaccine, it was clear that the protection level, measured in terms of percentage of relative protection (PRP) was comparatively high in IP vaccination than the IM one irrespective of the routes of challenge; after 4 weeks of vaccination . This could be attributed to oozing some of the immunizating dose of vaccine injected to fish by the muscular activity so that a larger doses of the antigen were required. This was also reported by Lamers et al., (1985a) who mentioned that maximum memory to *A. hydrophila* injected IM into carp was directly correlated with the antigen dose and the low primary dose induced only weak memory formation.

The application of the formalized whole culture vaccine by immersion of fish into the vaccine solution for different exposure me duration ; then challenged both by IP injection and immersion in a solution containing the living virulent isolate of *A.hydrophila* showed that immersion for 2 minutes provided the highest protection level. This could be attributed to the longer time of exposure of fish that permits their uptake of the vaccinal particles mainly by their gills and probably through skin and gut. The results supported those of Lamers et al., (1985 b).

Concerning the inoculation of common carp with formalized toxoid of *A.hydrophila* the results indicated high levels of protection of the IP and IM inoculated fish as proved by challenge with

the virulent homologous *A.hydrophila* 28 days post-immunization. These data indicated that the formalized ECPs (toxoid) was highly antigenic and could induce an effective protection against *A.hydrophila* isolates. These obtained results agree with those of Leung (1987).

In conclusion from the given data, the formalized whole culture vaccine of *A.hydrophila* appeared to be more effective and economic for controlling the MAS outbreaks among cultured common Carp in Egypt, particularly when it is prepared from the local *A.hydrophila* isolate.

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