

## Characterization And Pathogenicity Of *Vibrio Spp.* Contaminating Seafoods In Lagos, Nigeria.

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**Abstract:** Twenty-five seafood samples (shrimps, crabs and cuttle fish) collected from seven Fishing Companies and some local fishermen in Lagos, Nigeria, were examined for the presence of *Vibrio* species. A total of 44 vibrios belonging to five different species were isolated. The most predominant species was *Vibrio alginolyticus* (31.8%), followed by *V. harveyi* (27.3%), *V. mimicus* (22.7%), *V. parahaemolyticus* (11.4) and *V. cholerae* (6.8%). All the strains of *V. mimicus*, *V. parahaemolyticus* and *V. cholerae* and some strains of *V. alginolyticus* and *V. harveyi* lysed human red blood cells. None of the isolates tested elicited fluid accumulation in the experimental mice. However, histology sections showed that *V. parahaemolyticus*, *V. cholerae*, two strains of *V. mimicus*, one strain each of *V. alginolyticus* and *V. harveyi* caused erosion of the epithelial linings of the intestines of the experimental mice. This indicates that the organisms can cause infections in humans by invasion of the epithelial linings of the intestine.

**Key words:** Characterization, Pathogenicity, Seafoods, *Vibrio* species, Lagos, Nigeria.

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### Introduction

Vibrios are among the most common surface organisms in surface waters of the world. They occur in both marine and fresh water habitats and in associations with aquatic animals. Some species are pathogens of fish, eels and frogs as well as other vertebrates and invertebrates (Todar, 2005).

Species such as *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. mimicus*, *V. fluvialis*, *V. furnissii*, *V. metschnikovii*, *V. hollisae* and *V. damsela* are human pathogens (Levinson and Jawetz, 1996; Tison, 1999; Finkelstein, 2000). They account for a significant proportion of human infections such as gastroenteritis, usually associated with consumption of raw or undercooked seafood, wound infections, septicemia and ear infections (Baron *et al.*, 1994; Levinson and Jawetz, 1996; Tison, 1999). Most of these vibrios secrete enterotoxins in foods, water or in the gastrointestinal tract (Nishibuchi and DePaola, 2005).

In Nigeria, *V. parahaemolyticus* associated gastroenteritis due to consumption of contaminated seafood has been reported (Ndon, *et al.* 1992) as well as sporadic cases of cholera (WHO, 1991).

The aim of this study is to screen seafoods in Lagos for the presence of *Vibrio* species and determine their pathogenicity. The knowledge of this will help in the control of *Vibrio* – associated gastroenteritis in Nigeria as the awareness of the dangers associated with the consumption of raw or undercooked seafood will be created to the masses.

## Materials and methods

**Sample collection:** 25 seafood samples comprising shrimps, crabs and cuttle fish were collected from different Fishing Companies in Lagos while ten samples comprising shrimps and crabs were collected from local fishermen at Agboyi-Odo and Oworonsoki areas of Lagos. Samples were collected in sterile sample plates and transported in coolers. They were analyzed immediately after collection.

**Isolation of Bacteria;** The procedure of Elliot *et al.* (1998) was adopted. Alkaline peptone water (APW), pH 8.4, and Alkaline Peptone Salt Broth (APSB i.e. APW plus 3% NaCl), pH 8.4 were used for enrichment of the samples.

25 grams of the sample was weighed into 225ml of sterile APW and APSB each (i.e. 1:10 dilution) and blended with sterile warring blender. The APW homogenate was incubated at 37°C for 6 to 8 hours. After incubation, a loopful of the surface pellicle was subcultured on dried TCBS agar and streaked to obtain discrete colonies. The plate was incubated overnight (18 to 24 hours) at 37°C after which yellow colonies were subcultured on dried tryptic soy agar (TSA) supplemented with 2% NaCl (TSA-2%NaCl) and incubated overnight for further identification of the isolates.

For the APSB homogenate, the 3-tube MPN method was adopted (Elliot *et al.*, 1998). After incubation, a loopful of the surface pellicle from the bottles showing positive growth (turbidity) was transferred to dried TCBS agar plates and streaked to obtain discrete colonies. The plates were incubated overnight at 37°C, after which round, opaque, greenish and yellowish colonies were subcultured on dried TSA-2% NaCl plates and incubated at 37°C overnight (Elliot *et al.*, 1998). The isolates were subcultured into 1% tryptone broth supplemented with 3% sodium chloride (T<sub>1</sub>N<sub>3</sub>) and incubated at 37°C for 24 hours. The 24 hours broth cultures were stored at room temperature before identification

**Identification of isolates:** A loopful of each of the broth cultures stored at room temperature was transferred onto dried tryptic soy agar supplemented with 2% sodium chloride (TSA-2% NaCl) and streaked to obtain discrete colonies. The inoculated plates were incubated overnight at 37°C and the isolates were subjected to conventional biochemical tests (Barrow and Feltham, 1993; Elliot *et al.*, 1998) and confirmed with API 20E diagnostic strips.

**Test for pathogenicity:** Pathogenicity of the isolates was determined using tests for haemolysis and enterotoxin production.

**(i) Test for hemolysis:** A colony of each of the isolates was subcultured onto freshly prepared blood agar (nutrient agar containing human blood) plates and streaked to obtain discrete colonies. The plates were incubated at 37°C for 24 hours, after which the colonies were examined for hemolytic activity.

**(ii) Test for enterotoxin production:** The ability of the *Vibrio* isolates to produce enterotoxin was determined in infant mouse assay (Dean *et al.*, 1972). Two strains of *V. alginolyticus*, *V. harveyi* and *V. mimicus* and one strain of *V. cholerae* and *V. parahaemolyticus* were employed for the test.

**Infant mouse assay A:** The methods described by Merson *et al.* (1980) and Kothary and Richardson (1987) were adopted. Infant mice weighing between seven and twelve grams were used. Five colonies of each test isolate were subcultured into 5ml of tryptone broth with 3% NaCl (T<sub>1</sub>N<sub>3</sub>) and incubated at 37°C for 24 hours. After incubation, the broth cultures were centrifuged at 4000 r.p.m for 15 minutes. The supernatant of each isolate was decanted into a sterile test tube. 0.3ml of the supernatant of each culture was orally fed into nine infant mice (just weaned) weighing between 7 and 12 grams. Two negative controls were set up. For control 1, nine mice were left unfed while for control 2, nine mice were orally fed with 0.3ml of T<sub>1</sub>N<sub>3</sub> broth. All the mice in each group were held at room temperature. After 2, 4 and 6 hours consecutively, three mice from each group were anaesthetized with chloroform and dissected. The complete intestine, stomach and the remaining body of each mouse were separately weighed to determine their fluid accumulation ratio.

**Infant mouse assay B:** Suckling mouse assay method of Kothary and Richardson (1987) was adopted. Suckling mice weighing between three and five grams were used for the assay. Five colonies of each of the test isolates were subcultured into 5ml of brain heart infusion broth containing 2% NaCl and incubated at 37°C for 24 hours. Thereafter, the broth culture was centrifuged at 4000 r.p.m for 15 minutes. The cells obtained were washed twice with 10ml of phosphate buffered saline (PBS) [0.02M Na<sub>2</sub>HPO<sub>4</sub>, 0.15M NaCl, pH 7.4] and suspended in

the same buffer (PBS). Suckling mice (three per group) were orally fed with 0.2ml of the cells suspended in PBS. Three mice in the control group were orally fed with 0.2ml of PBS. All the mice were held at room temperature for 4 hours. Thereafter, the mice were anaesthetized with chloroform and dissected. The complete intestines, stomach and the remaining body of each mouse were separately weighed for determination of the FA ratio for each mouse.

**Determination of fluid accumulation (FA) ratio.** The FA ratio for each mouse was determined as described by Baselski *et al.*, (1977). The FA ratio was expressed as 1000 times the ratio of the weight of the stomach plus the intestine to the remaining weight.

**Histology:** Portions of the intestines of the experimental mice were cut to blocks of 5x3x2cm, processed and stained using haematoxylin and eosin technique. The photographs of the stained sections were taken with Reichert Microstar IV photomicroscope using colour film 135m.

## Results

**Isolation and characterization of isolates:** Table 1 shows the occurrence of the *Vibrio* species in the seafood samples. *V. alginolyticus* 14(31.8%) had the highest occurrence frequency in all the samples. This was followed by *V.harveyi* 12 (27.3%), *V. mimicus* 10(22.7%), *V. parahaemolyticus* 5(11.4%) and *V.cholerae* 3(6.8%).

### Pathogenicity

**(i) Hemolysin production:** 5 strains of *V. alginolyticus* lysed human red blood cells while 9 strains did not cause hemolysis. Also 6 strains of *V. harveyi* lysed human red blood cells while 6 strains did not. On the other hand, all the strains of *V. parahaemolyticus*, *V. mimicus* and *V. cholerae* screened lysed human red blood cells on blood agar (Table 2).

**ii) Enterotoxin production:** The fluid accumulation (FA) ratios of the *Vibrio* isolates are shown in figures 1 and 2.

**Assay A:** Figure 1 shows that after 2, 4 and 6 hours incubation, there were no marked differences in the fluid accumulation ratios of the two negative controls and the 8 *Vibrio* species

screened for enterotoxin production. Also, statistical analysis [Analysis of Variance (ANOVA)] of the fluid accumulation ratios of the two negative controls and the eight *Vibrio* species screened showed no significant difference between all the FA ratios after two, four and six hours at 5% level of significance.

**Assay B:** The FA ratios of phosphate buffered saline (PBS) and the *Vibrio* isolates were almost at same level after four hours incubation (Figure 2).

Statistical analysis [Analysis of Variance (ANOVA)] of the fluid accumulation ratios of the negative control and the eight *Vibrio* species also showed no significant difference between the FA ratios of PBS and the eight *Vibrio* isolates after four hours incubation at 5% level of significance.

**Histology:** Histology sections showed that the intestines of the infant mice fed with *Vibrio cholerae*, the first strain of *Vibrio alginolyticus* (VA1) and *Vibrio harveyi* (VH1) still remained intact after six hours incubation (Plate 1). On the other hand, the intestines of infant mice fed with the second strain of *Vibrio alginolyticus* (VA10), the two strains of *V. mimicus* (VM1 and VM5), the second strain of *V. harveyi* (VH7) and *Vibrio parahaemolyticus* (VP1) showed significant erosion of the epithelial linings (Plate 2).

In the case of the suckling mice used in assay B, histology sections showed that the intestines of the suckling mice fed with the first strain of *Vibrio alginolyticus* (VA1) and *Vibrio harveyi* (VH1) were still intact after four hours incubation (Plate 3). However, the intestines of those fed with *V. cholerae* (VC1), *V. parahaemolyticus* (VP1), the two strains of *V. mimicus* (VM1 and VM5), the second strains of *Vibrio alginolyticus* (VA10) and *V. harveyi* (VH7) had their epithelial lining significantly eroded (Plate 4). The summary of the reactions of the eight *Vibrio* species screened on the epithelial linings of the intestines of infant mice is shown in table 3.

## Discussion

In this study, a high frequency of occurrence of *V. alginolyticus* and *V. harveyi* was observed in the seafoods analyzed. Similar observations had been made by Elhadi *et al.* (2004) in studies conducted in Malaysia and in Morocco by

Bouchriti and El Marrakchi, (1995). Elsewhere, high occurrence of *V. alginolyticus* has also been reported in Indonesia by Molitoris *et al.* (1985). This high prevalence may be attributed to their high salt tolerance ability. In contrast, Caldini *et al.* (1997) reported that out of 150 *Vibrio* species isolated from the Arno River basin in Italy, *V. cholerae* non-O1 was the most prevalent species with 82% occurrence. This was attributed to the fact that the River basin is a freshwater.

The *Vibrio cholerae* strains isolated in this study lysed human red blood cells on blood agar but did not produce cholera toxin (which induces fluid accumulation in infant mice). Previous study by Barrow and Feltham (1993) showed that clinical strains of *V. cholerae* O1 and non-O1 produce cholera toxin while their environmental strains do not.

Similarly, the two strains of *V. alginolyticus* screened did not induce fluid accumulation in the experimental mice. Also, only 5 out of 12 strains isolated in this study lysed red blood cells on blood agar. This is similar to the findings of Molitoris *et al.* (1985) who observed that out of five environmental strains of *V. alginolyticus* screened for hemolysin production, only one strain was positive. A similar trend was also observed with the strains of *V. harveyi* isolated in this study.

All the strains of *V. mimicus* (10(22.7%)) isolated in this study lysed human red blood cells but did not induce fluid accumulation in the experimental mice. Chowdhury *et al.* (1987) had earlier reported that out of 125 environmental strains of *V. mimicus* isolated from aquatic environments of Bangladesh, only one strain induced fluid accumulation in suckling mice. Our finding is in line with this, the only difference is the number of organisms screened.

In this study, it was discovered that the *V. parahaemolyticus* strains isolated from the seafood samples lysed human red blood cells on blood agar but neither the culture filtrate nor the whole cells induced fluid accumulation in the experimental mice. Previous investigation conducted by Kaysner and DePaola (2004) had shown that most clinical strains of *V. parahaemolyticus* produce enterotoxigenic hemolysin while the environmental strains do not.

Histology examination of the intestinal architecture of the experimental mice when fed with the test organisms showed that with the exception of one strain each of *V. harveyi* and *V. alginolyticus*, the other six strains (one strain of *V. cholerae*, two strains of *V. mimicus* and one strain of *V. parahaemolyticus*) caused erosion of

the epithelial lining of the intestines of the experimental mice. This means that the *Vibrio* species isolated from this environment could be invasive but not enterotoxigenic since they did not elicit fluid accumulation in the experimental mice. Similarly, previous studies by Baron *et al.* (1994) and Todar (2005) had established that *Vibrio* species such as *V. parahaemolyticus* and *V. vulnificus* are invasive in nature.

This study shows that *Vibrio* species are common in seafood caught in Lagos aquatic environment and although these species may not be enterotoxigenic, they could cause infections in humans by invasion of the epithelial linings of the intestines. Thus, thorough adequate cooking of seafoods sold in Lagos is recommended.

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**Table 1:** Occurrence of *Vibrio* species in the seafood samples from seven fishing companies and catches from local fishermen at Agboyi-Odo and Oworonsoki in Lagos.

ISOLATES	NUMBER OF ISOLATES									
	C1	C2	C3	C4	C5	C6	C7	A	O	TOTAL
<i>Vibrio alginolyticus</i>	2	0	3	1	1	1	1	3	2	<b>14(31.8%)</b>
<i>Vibrio parahaemolyticus</i>	1	3	0	0	0	1	0	0	0	<b>5(11.4%)</b>
<i>Vibrio mimicus</i>	0	2	1	1	0	1	1	3	1	<b>10(22.7%)</b>
<i>Vibrio harveyi</i>	2	1	2	0	2	1	1	2	1	<b>12(27.3%)</b>
<i>Vibrio cholerae</i>	0	0	0	0	0	0	0	1	2	<b>3(6.8%)</b>
<b>TOTAL</b>	<b>5</b>	<b>6</b>	<b>6</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>3</b>	<b>9</b>	<b>6</b>	<b>44(100%)</b>

- C=Company; A=Agboyi-Odo; O=Oworonsoki

**Table 2:** Hemolysin production by the *Vibrio* species isolated from seafoods in Lagos

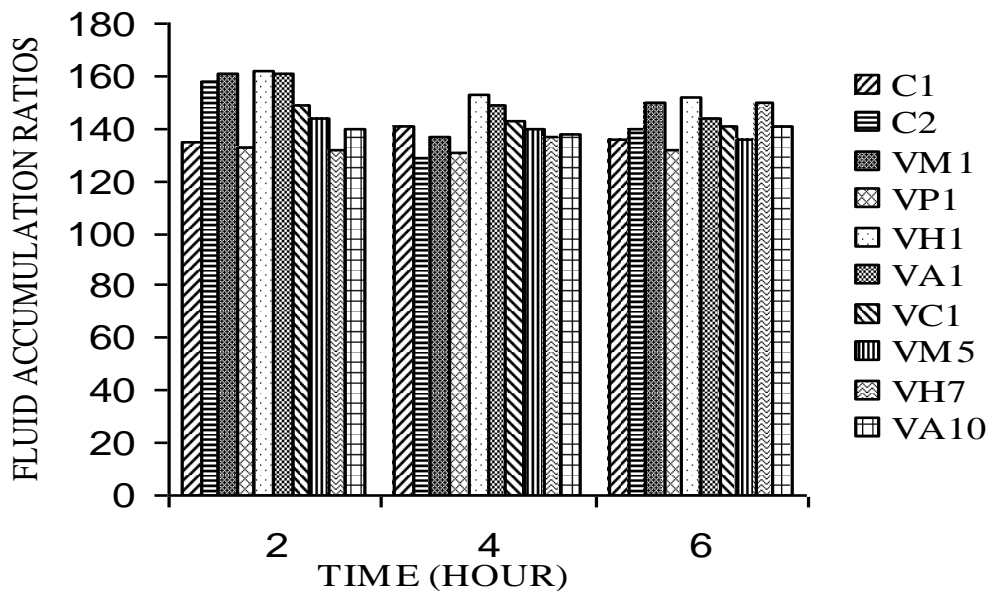
ISOLATE	HEMOLYSIN PRODUCTION
VA (1-9)	-
VA (10-14)	+
VP (1-5)	+
VH (1-4)	-
VH (5-6)	-
VH (7-12)	+
VM (1-10)	+
VC (1-3)	+

\* + = positive; - = negative

**Table 3:** Invasiveness of *Vibrio* species isolated from seafoods in Lagos on the epithelial linings of the small intestines of infant mice.

ISOLATE	ASSAY A	ASSAY B
VC (1)	-	+
VA (1)	-	-
VA (10)	+	+
VH (1)	-	-
VH (7)	+	+
VM (1)	+	+
VM (5)	+	+
VP (1)	+	+

- + = erosion of epithelial lining,  
- = non-erosion of the epithelial lining  
ASSAY A/ASSAY B = Suckling/or infant mouse assay



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Figure 1

C1 (Control 1)= Mice not fed; C2 (Control 2)= 1: Comparison between FA ratios of eight *Vibrio* species and two negative controls in infant mice (7-12g). FA ratios were determined for three mice per p. (Values represent mean FA ratios). 1% tryptone broth +3% NaCl;  
VM= *Vibrio mimicus*; VP= *Vibrio parahaemolyticus*; VH= *Vibrio harveyi*;  
VA= *Vibrio alginolyticus*; VC= *Vibrio cholerae*

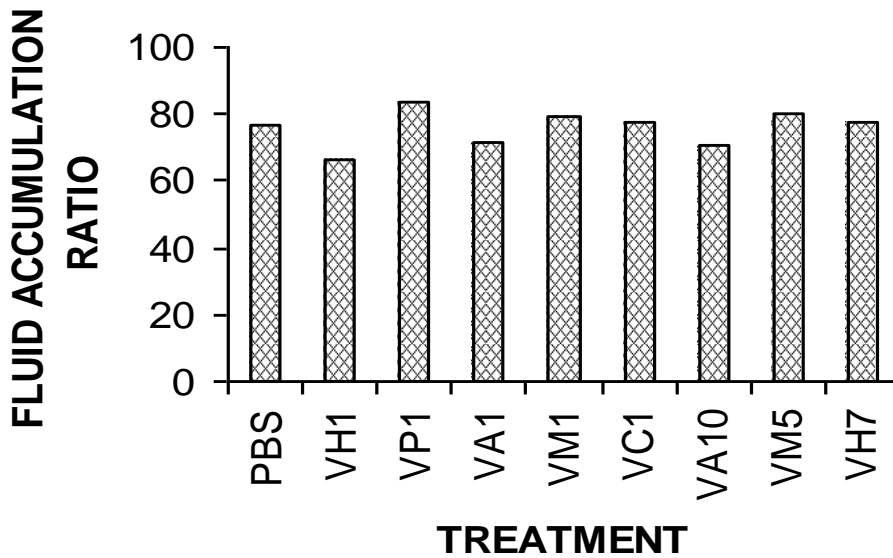


Figure 2: Comparison between the mean fluid accumulation (FA) ratios of phosphate buffered saline (negative control) and eight *Vibrio* isolates in suckling mice (3-5g) after four hours incubation. FA ratios were determined for three mice per group. (Values represent mean FA ratios).

PBS= Phosphate buffered saline; VP= *Vibrio parahaemolyticus*; VH= *Vibrio harveyi*; VA= *Vibrio alginolyticus*; VC= *Vibrio cholerae*; VM= *Vibrio mimicus*(Plate 2).

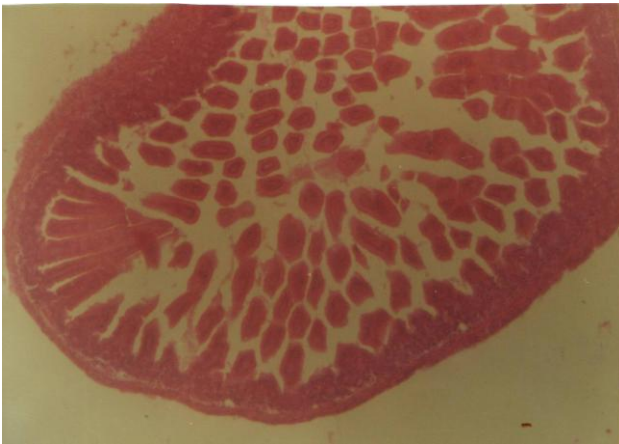


Plate 1: Histological section of the intestine of an infant mouse fed with *Vibrio alginolyticus* (VA1).

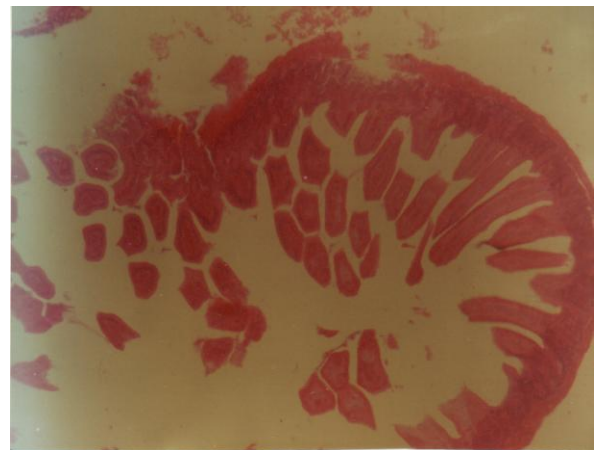


Plate 2: Histological section of the intestine of an infant mouse fed with *Vibrio parahaemolyticus* (VP1).

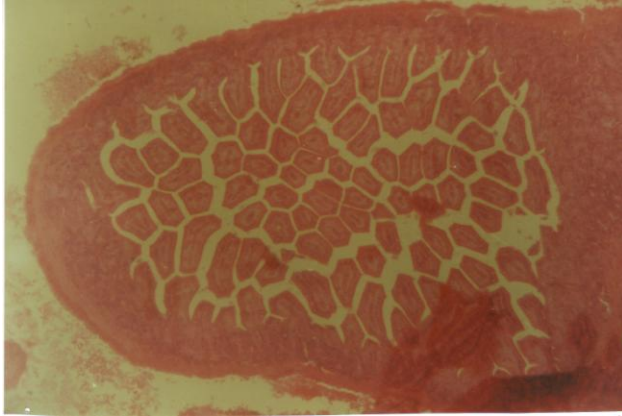


Plate 3: Histological section of the intestine of a suckling mouse fed with *Vibrio harveyi* (VH1).

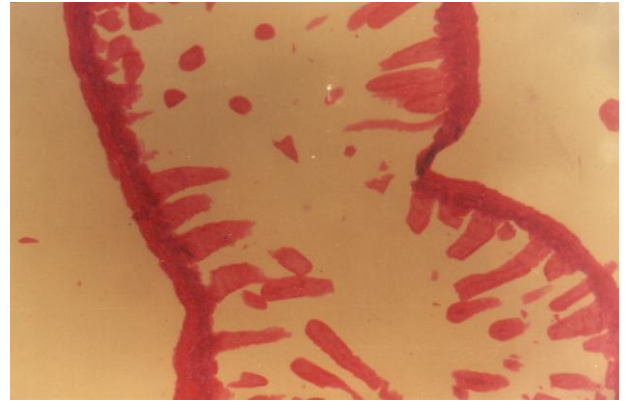


Plate 4: Histological section of the intestine of a suckling mouse fed with *Vibrio cholerae* (VC1).