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Original Paper

Histopathological Changes Associated with Experimental Infection of *Arcobacter butzleri* in Albino Rats

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ABSTRACT

Arcobacters are emerging food borne pathogens potentially associated with prolonged diarrhoea and occasional systemic infections but the pathogenic mechanisms of these bacteria are largely unknown. This study was designed to investigate the pathogenicity of *Arcobacter* isolates. Two strains of *A. butzleri* isolated from stool of healthy chickens were confirmed with real time PCR and tested on albino rat by giving a single oral challenge of 10^9cfu/ml to 65 healthy adult male rats. Five (5) uninfected animals were used as control. Diarrheoal illness occurred in all rats from the fifth day and resolved from day 21 post infection, severe histopathological lesion such as hepatic necrosis, villous erosion, desquamation, matting and necrosis of the segments of small intestine was also observed. In this study, the toxic ileitis necrosis pattern of pathology in the gut of experimentally infected rats could be an indication of observed persistent watery diarrhea associated with the clinical presentation of *Arcobacter* infection in humans. The pathology of *A. butzleri* in albino rats had not been previously described, and it appears that the present study is the first report in Nigeria. It may therefore be useful for further investigation.

Keywords: Albino rats, *Arcobacter*, Experimental infection, Histopathology, Toxic ileitis necrosis, Nigeria.

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INTRODUCTION

Arcobacter infection in domestic animals is associated with spontaneous abortion, diarrhea, mastitis, on the other hands the spectrum of infection in humans is dominated by gastroenteritis and occasionally, extra intestinal manifestation; septicemia, endocarditis, arthritis, peritonitis, liver cirrhosis (Lastovica and Skirrow, 2000). In an observational study, *A. butzleri* was described to display similar clinical and microbiologic features with *C. jejuni.* However, patients with *A. butzleri* report diarrhoea associated with abdominal pain; nausea and vomiting or fever and were more likely to have persistent diarrhoea than those with *C. jejuni* infection (Vandenberg *et al.,* 2004). There have been a few animal studies on the pathogenicity of *Arcobacter* species. Experimental oral infection of caesarean-derived 1-day-old piglets showed that strains colonized and multiplied in the gut but only *A. butzleri* strains (from human faeces and swine) were able to invade the internal organs of infected animals. The mortality due to the *A. butzleri* and *A. skirrowii* strains in the first trial was not observed when the experiment was repeated and the study concluded that variable results obtained could be explained by different susceptibility of the piglets and their age (Wesley *et al.,* 1996).

In another study, where 3-5-day-old chickens and turkeys were infected orally with the human *A. butzleri* strains, the human strain could not colonize and invade the White Leghorn chickens and commercially out-bred turkeys, but was recovered from cloacal swabs and tissues of highly inbred Beltsville White turkeys.

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The results showed that the invasive capacity and virulence of the *A. butzleri* strains were host-dependent with respect to species and breed (Wesley and Baetz, 1999). Recently, the pathological effects of *Arcobacter cryaerophilus* intramuscular infection in 40 healthy 1- year old rainbow trout (*Oncorhynchus mykiss Walbaum*) was reported to cause deaths with gross clinical abnormalities such as degenerated opercula, gills, liver damage, haemorrhagic kidneys, serous fluid in swollen intestines and significant reduction in the red blood cell count, serum cholesterol and total protein in the blood (Yildiz and Aydins, 2006). Albino rat (*Rattus novegicus)* is an important model animal in biological research that has been used extensively to study biological phenomenon, with the expectation that lesions produced will provide an insight to course of oral infection of *Arcobacter* human host.

To the best of our knowledge, the pathology of *Arcobacter* infection in albino rat has not been studied and because of its zoonotic importance, this study therefore aims to document the histopathological lesions associated with oral infection of *Arcobacter* in adult rat with the view of obtaining insights to its pathogenicity.

MATERIALS AND METHODS

Identification of bacteria

Conventional isolation methods of *Arcobacter* were carried out as described by Vandamme *et al*., (1992). Molecular characterization and confirmation was done by real time PCR procedure targeting the gyrase A subunit gene outside the quinolone resistance determining region developed to detect *Arcobacter* species.

Animals

Sixty-five (65) male five-months-old healthy albino rats (*rattus novegicus*) weighing 200-250g were acquired from the animal house unit of College of Health Sciences, LAUTECH, Osogbo, Nigeria. They were housed in transparent plastic cages of dimensions 33cm x 20.5 cm by 19 cm and were allowed to be accustomed to the new environment and human handling. The animals were fed on antibiotics free ration and given water *ad libitum.* They were grouped into 5 rats per cage each group received 10^9 CFU per ml. The control group received sterile normal saline and tagged before inoculation. Feacal culture of the rats was done to rule out previous infection with *Arcobacter* organism.

Preparation of *Arcobacter* inocula

Strains of *Arcobacter* maintained in glycerol *Arcobacter* broths (Oxoid^R) at -25^{o}C were resuscitated in brain heart infusion agar supplemented with 5% yeasts and 7% sheep blood and incubated at 35-37^{o}C in microaerophilic atmosphere (Vandamme *et al*., 1992). The bacterial culture was suspended in 0.95% normal saline and standardized by McFarlands Nephelometry of 10^9 CFU per ml.

Animal Inoculation

One ml of A. *butzleri* suspension containing 10^9 CFU (colony forming units) was given orally to the rats with 1ml sterile syringe. 1ml of sterile normal saline was given to the five (5) rats to control the experiment.

Macroscopic lesion scoring

Viscera and abdominal organs were dissected and staked with pins. This was viewed with magnifying glass and examined for visible gross pathological changes.

Histopathology

Animals were sacrificed on day 5, 8, 14 and 21 of the experiment after bacterial inoculation. Organs specimens from the liver and various sections of the intestine of the rats were fixed in 10% formalin for 24 hours, embedded in paraffin sections stained with Haematoxylin and Eosin (H&E) and examined microscopically for histopathological changes. The villous/crypt ratio, the villous height, crypt depth and number of crypts per villous were assayed by random measurement of 10 villi/crypts per section (one section per gut region per rat) using a PC-based image analysis system (Olympus B x 61 Digital camera dP50; Olympus NV, Belgium with software analysis^R J2). The villus length/crypt depth (V/C) ratio was determined and the mean was calculated for each gut segment and for each test group.

RESULTS

Table 1: Histopathological Changes in Small Intestines of *Arcobacter*-infected rats

Degree of pathologic changes										
Microscopic findings	day 5		day 8		day 14		day 21			
	J	I	J	I	J	I	J	I		
Changes in villi										
Blunting	-	-		≠	≠	≠	≠	≠	≠	
Matting	-	✔	✔	✔	✔	✔	✔	≠		
Thickening	-		-	+	+	≠	-	✔	✔	
Adhesion	+		+	≠	≠	≠	✔	✔	✔	
Atrophy			+	+	≠	✔	✔	✔	✔	✔
Tip Erosion			+	+	✔	✔	✔	✔	✔	✔
Loss of villi			+	+	+	-	+	✔	✔	✔
Stromal changes										
Edema	+		+	✔	✔	✔	✔	✔	✔	
Congestion			+	+	+	+	=	≠	✔	✔
Cell infiltration	+		+	✔	✔	✔	✔	✔	✔	
Fibrosis	-		-	-	+	+	+	+	+	
Crypt changes										
Epithelial hyperplasia	+		+	+	≠	≠	≠	≠	≠	
Villous/ crypt ratio	1/4		1/3	1/3	1/3	1/3	1/3	I/3	1/3	

Legend: _ = No changes, + = Slight changes, ≠ = Moderate changes, ✔ = Marked changes, J= Jejunum, I= Ileum

Reduced activity, reduced appetite, rough coat and obvious diarrhoea were observed in the infected group. The diarrhoeic stool was loose, containing mucus but no blood was seen with or without microscope. Diarrhoea appeared to be self limiting after 6 weeks without therapeutic intervention. The control groups were healthy throughout the period of experiment. The intestinal blood vessels were markedly hyperaemic and moderate oedema was observed in the mucosa compared with the uninoculated epithelium (Figure 1).

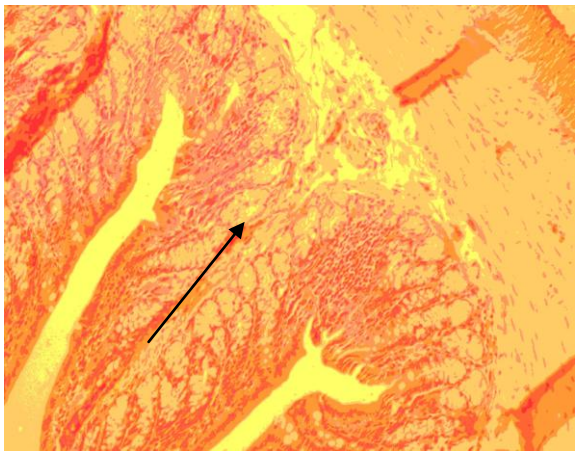
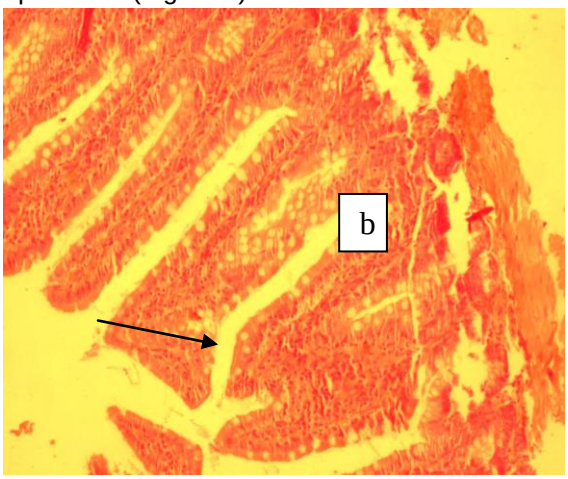


Figure 1: Histological longitudinal section of an uninoculated ileum showing intact villi (Bright field microscopy X100): Negative control.



Arrow: 'pinching off of projections' of the villus of ileo-caecal junction and (b) circular whitish lining the projections (Light bright field microscopy X100)

Figure 2: Longitudinal section of tip erosion goblet cells of *Arcobacter butzleri* infected rat.

Degenerative changes at various sections of intestine include erosion (Figure 2), matting thickening (Figure 3), atrophy, villous desquamation (Figure 4) and stunting of villi with mild hemorrhage were observed in *Arcobacter* inoculated rats. The villous and crypt ratio were reduced and cryptal cells hyperplasia was also observed. The mucosa was infiltrated with neutrophils and cytoplasm vacuolar degeneration (fatty change) was observed as evidence of toxicity in the liver (Figure 5). In the gut of rats inoculated with *Arcobacter*, there was marked necrosis of the villi (Figure 4), infiltration of leukocytes into the lamina propria (A). The intestinal blood vessels were markedly hyperaemic and moderate oedema was observed in the mucosa compared with the uninoculated epithelium.

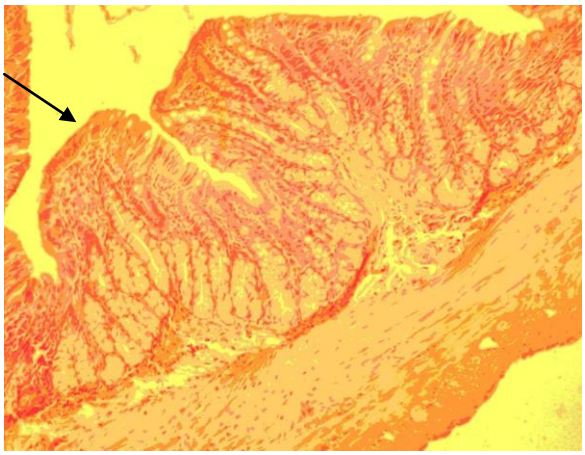


Figure 3: Illeum section of light bright field microscopy showing stunting and matting of villi of *Arcobacter butzleri* infected rats (X100)

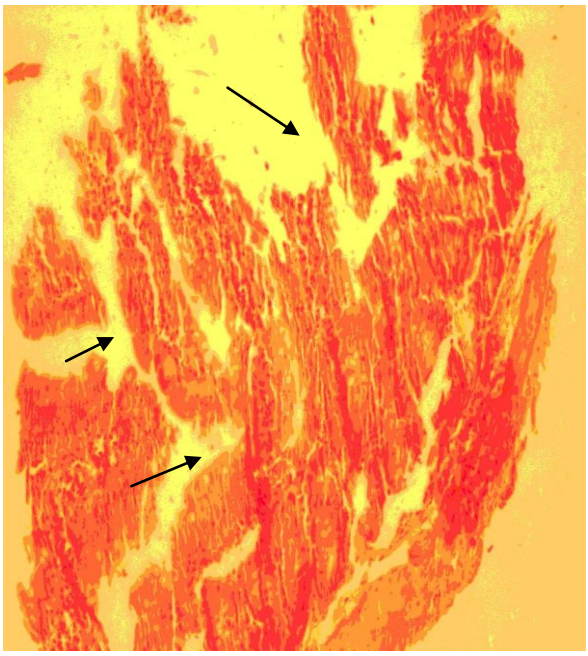


Figure 4: jejunum showing marked villous desquamation and necrosis in *Arcobacter butzleri infected* rat (bright field microscopy X100)

The morphological diagnosis was therefore acute toxic ileitis. Other changes in jejunum and ileum on day 5, 8, 14 and 21 at 10^9 cfu/ml of *Arcobacter* post inoculation are summarized on Table 1.

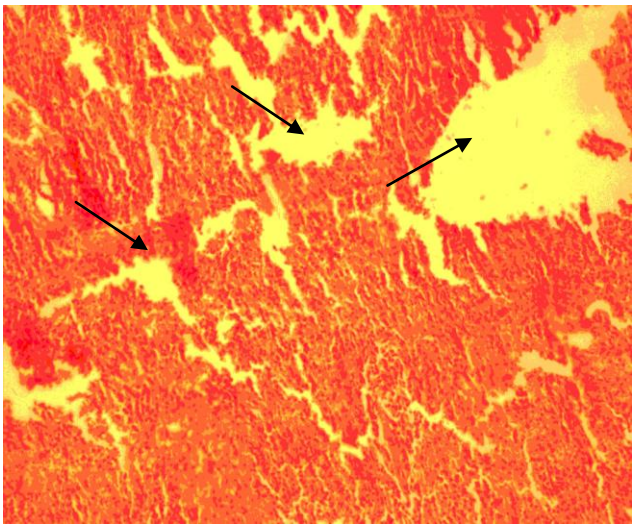


Figure 5: Liver sample of inoculated rat showing disruption of lobular architecture and necrosis of hepatocytes indicating toxicity (bright field microscopy X100).

DISCUSSION

Experimental infection of *Arcobacter* in albino rats had been previously established during a pilot study earlier in this experiment (data not shown) to obtain (infective dose) of ID50 for 10^3 cfu/ml *Arcobacter* organism. 10^3cfu per ml of bacteria produced mild and 10^9 produced significant pathological changes in male albino rats which provide the basis for the dose of 10^9 used in this experiment. Marked histopathological features observed in this study are clear indications of the pathogenic capabilities of Arcobacters in albino rats. It could therefore be inferred that rat is a useful and a sensitive model for studying the effect of oral exposure to *Arcobacter* over a wide dose.

Histological lesion such as disruption of cytoskeletal structure of the ileum, marked necrosis, desquamation, stunting, matting and

atrophy of the villi, goblet cell hyperplasia were clear evidence of the toxigenic potentials observed in a study (Fernandez *et al.,* 1995). The observation possibly indicates evidence of adherence factors and colonization of the intestine by pilation process described as ability of bacteria to adhere to entero-receptor sites on specific cells surface thereby enabling the organism to colonize the intestine (Carbone *et al*., 2003).

Also in this study, following a successful infection, the appearance of generalized cytoplasmic vacuolar degeneration (fatty change) in the liver of rats after diffusion of toxins from the ileum showed evidence of toxicity. This observation also corroborates with reports from a previous study that demonstrated the detection and cytotoxic effects of *Arcobacter* on INT cell lines (Villarruel-Lopez *et al*., 2003). In a similar study where enteropathogenicity of *Arcobacter* strains isolated from human and animal sources was detected using ligated loop of rat, accumulation of fluid and hyperemic loop was demonstrated (Musmanno *et al*., 1997).

In this study, for adult rats the infective dose of 10^9 strains was sufficient to produce diarrhea in the challenged rats. The organism persisted in the digestive tract of the rats till about four weeks post infection. This observation was similarly reported in another study which postulated that the persistence might be due to the adhesion brought about by interactions between the bacteria and the host intestinal mucosa (Vanderberg *et al*., 2005). A clearance of the bacterial was observed as the weeks ran by (> 4 weeks). This was inferred from the result obtained from lesions obtained from 5 to 21 days in table 1 where degree of severity of lesion reduced as the days passed-by. This might be a phenomenon usually observed in natural infection of subject exposed to infection especially in immunocompetent host that is naturally endowed with ability to fight infection as the case in self limiting diseases (Ho et *al*., 2006).

It is also noteworthy that there was no mortality due to the experimental inoculation of Arcobacter except those that died as a result of ether overdose during blood collection during the pilot study (data not shown). In subsequent experiment, caution was taken to dose the rats lightly to prevent death of rats. In conclusion, the findings from histopathological features observed in this study suggest that Arcobacters are pathogenic in albino rats and can also be considered a suitable animal model for its experimental studies.

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