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Effects of Food Borne Pathogens on the Internal Organs of Albino Rats (*Rattus norvegicus*)

Ibikunle I. Anibijuwon[1]*, Anthony K. Onifade[2], Moshood K. Oladumoye[2]

[1]Public Health Laboratory Unit, Department of Microbiology, Faculty of Science, University of Ilorin, PMB 1515, Ilorin, Kwara State, Nigeria
[2]Department of Microbiology, Federal University of Technology, PMB 504 Akure, Ondo State, Nigeria

*Corresponding author

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Abstract

Different bacterial and fungal species were isolated from ready-to-eat foods in six major cities in Ondo State, Nigeria. The pathogenicity testing was carried out using albino rats which were fed with food-borne pathogenic isolates. The feeding process was carried out for a period of two weeks, during which the weights and physical appearance of the rats under study were closely observed and recorded. The internal organs of experimental rats with special emphasis on heart, liver, kidney, small and large intestines were also used as case studies. Albino rats were fed with feed contaminated with microbial isolates of food origin. The organisms are *Escherichia coli*,

Pseudomonas aeruginosa, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Bacillus cereus* and *Salmonella typhi*. The rats were grouped into seven categories with each containing two rats which were fed with a specific pathogen. The results for hematology test showed that infected rats had an increase in white blood cell count in relation to their PCV while the uninfected rats (control rats) had normal white blood cell counts in relation to their PCV. Histopathology tests showed that infected rats demonstrated hemorrhage destruction and aggregation of lymphoid cells in the livers and heart. Uninfected rats showed intact tissues in their livers and hearts. It can however be deduced that the effects of food-borne pathogens is cytolytic in the tissues and there is a geometric increase in white blood cells activated in response to invasion of food-borne pathogens.

Keywords: Cytolytic, foodborne, hemorrhage, foodborne pathogens, internal organs.

Introduction

Food-borne infections are caused by pathogens or their toxins and it is an important global public health threat. The World Health Organization [1, 2] also described it as the resultant effect of the consumption of contaminated food or water. Most cases of food poisoning or infection are caused by a variety of pathogenic microbes that contaminate food [2].

During the incubation period, microbes migrate into the intestine through the stomach, attach to the intestinal wall lining and replicate. Some microbes remain in the intestine, while some produce toxins that is dispersed into the bloodstream and some can directly invade body tissues. The symptoms produced depend on the type of microbe. Symptoms may begin within hours to several days after consumption. General manifestations include: nausea, abdominal pain, vomiting, diarrhea, gastroenteritis, fever, headache or fatigue [3]. Foodborne diseases can result fatal health issues or even death, especially for people at high risk. Most frequently isolated food-borne pathogens include *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Bacillus cereus* and *Salmonella typhi*.

Mice are suitable models for experiments in ascertaining the effect of biotic factors on humans. This study therefore used albino rats as model to quantify the effect of foodborne pathogens on internal organs.

Materials and Methods

Source of organisms

During the preliminary investigation of ready to eat food samples, pathogens of food borne origin were isolated, identified as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Bacillus cereus* and *Salmonella typhi*. The stock culture of these isolated organisms from food samples were used in this study.

Experimental design and treatment of animals

Thirty male albino rats, weighing 40g to 50 g, were received from the Department of Animal Sciences, Federal University of Technology, Akure, Nigeria. They were randomly assigned to six groups of five animals each. After 7 days habituation on the basal diet, the albino rats were randomly divided into control and treatment groups A, B, C, D, E and F. The animals were assigned randomly into separate cages and introduced to diets compounded with the foodborne pathogens under study for two weeks.

Animals were sacrificed by anaesthetizing with diethyl ether and venous blood was drawn by cardiac puncture and organs were blotted on filter paper and weighed. They were fixed in formal saline for histopathological studies.

Pathogenicity testing of the microorganisms

Pathogenicity testing of the microorganisms was performed using the method described by Liu [4]. Animal models have been used widely and data are converted to approximately the human form of the disease. In the animal model, route of infection, tissue distribution and degree of virulence should be equivalent to human infection.

Results

During the preliminary investigation, the following microorganisms were isolated: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Bacillus cereus* and *Salmonella typhimurium*, *Aspergillus niger*, *Aspergillus parasiticus* and *Aspergillus flavus*, *Mucor mucendo* and *Penicillium notatum*, although more than one specie was isolated from two or more food substances. The histopathology of various organs of the animal used manifested various damages ranging from hepatic drainage, hemorrhage formation to aggregation of lymphoid tissues.

The histopathological test:

Figure 1a: Liver of untreated rat that served as **control** experiment with cells remaining intact.

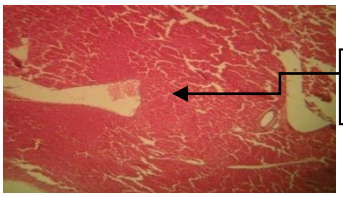

Figure 1b: Liver of rat fed with *Klebsiella pneumoniae*, showing highly affected hepatic drainage

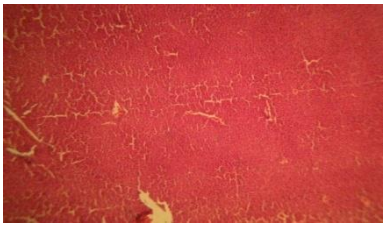

Figure 2a: Liver of rat that served as **control** experiment with cells remaining intact.

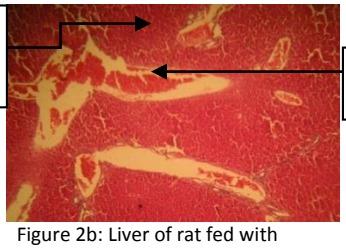

Figure 2b: Liver of rat fed with *Pseudomonas aeruginosa* showing hemorrhage formation and aggregation of lymphoid tissue.

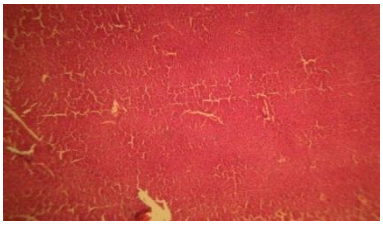

Figure 3a: Liver of rat that served as **control** experiment with cells remaining intact.

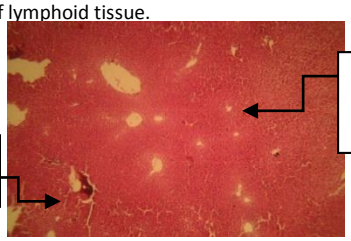

Figure 3b: The liver of rat fed with *Staphylococcus aureus* showing high hepatic drainage and an aggregation of lymphatic cells.

Figure 4a: Control experiment

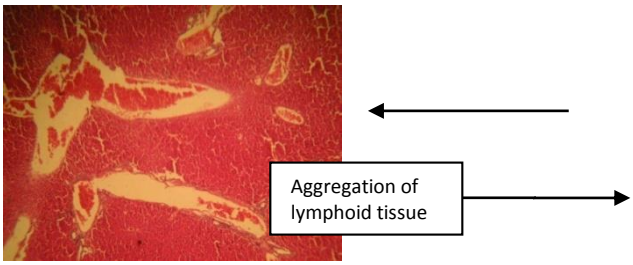

Figure 4b: Liver of rat fed with *Ps. aeruginosa* showing Hemorrhage formation and aggregation of lymphoid tissue.

Figure 5a: Liver of rat that served as **control** experiment with cells remaining intact.

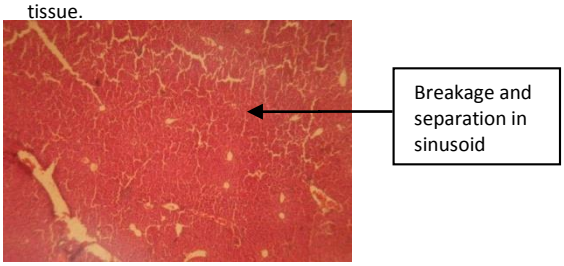

Figure 5b: Liver of rat fed with *E.coli* showing a wide separation in sinusoid.

Figure 6a: Liver of rat that served as **control** experiment with cells remaining intact.

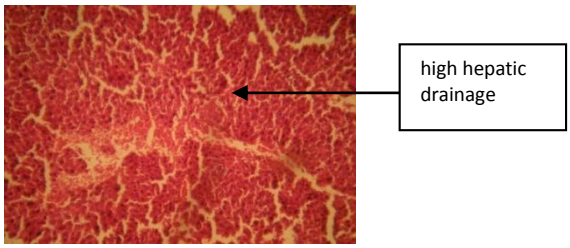

Figure 6b: Liver of rat fed *Klebsiella pneumoniae*, demonstrating an highly affected hepatic drainage

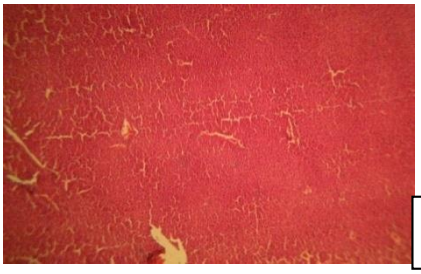

Figure 7a: Liver of rat that served as **control** experiment with cells remaining intact.

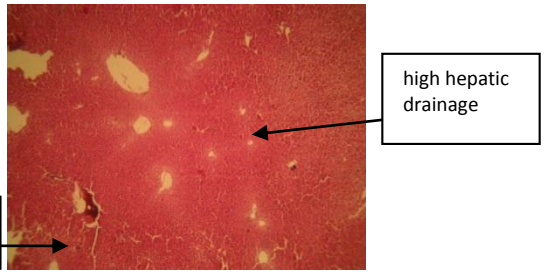

Figure 7b: The liver of rat fed with Staphylococcus *aureus* showing an aggregation of lymphatic cells

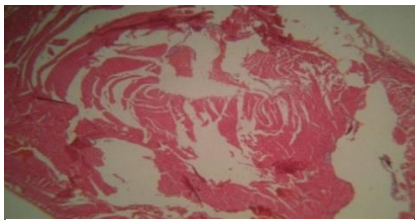

Figure 8a: Heart of untreated rat that served as **control** with cells of cardiac muscles remaining intact

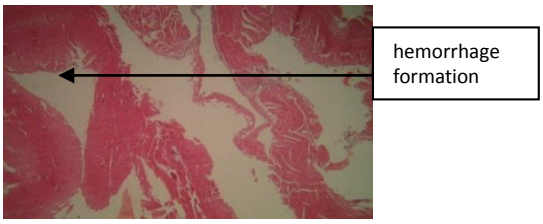

Figure 8b: Heart of rat fed with *Klebsiella pneumoniae* with hemorrhage highly noticed.

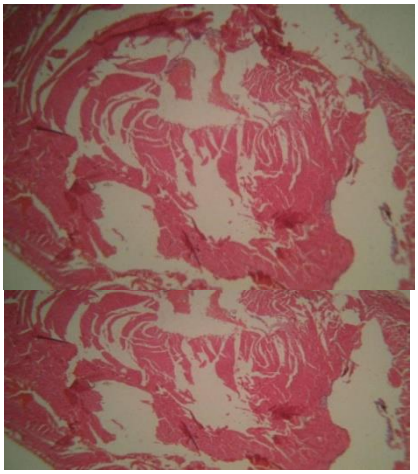

Figure 10a: Heart of untreated rat that served as **control** with cells of cardiac muscles remaining intact

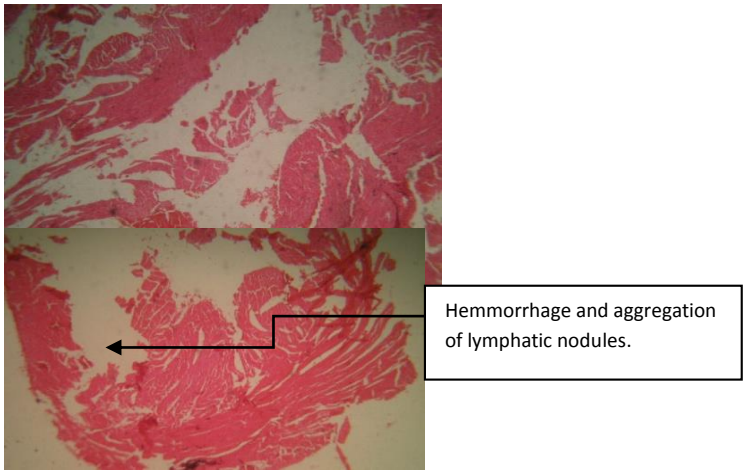

Figure 10b: The heart of rat fed with *Staphylococcus*with an evidence of hemorrhage and little aggregation lymphatic nodules.

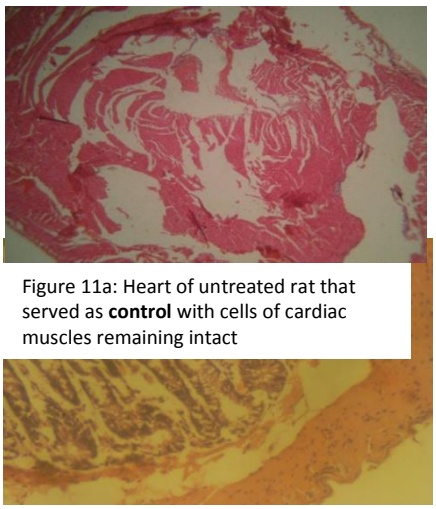

Figure 11a: Heart of untreated rat that served as **control** with cells of cardiac muscles remaining intact

Figure 12a: A part of the small intestine of the rat used as control.

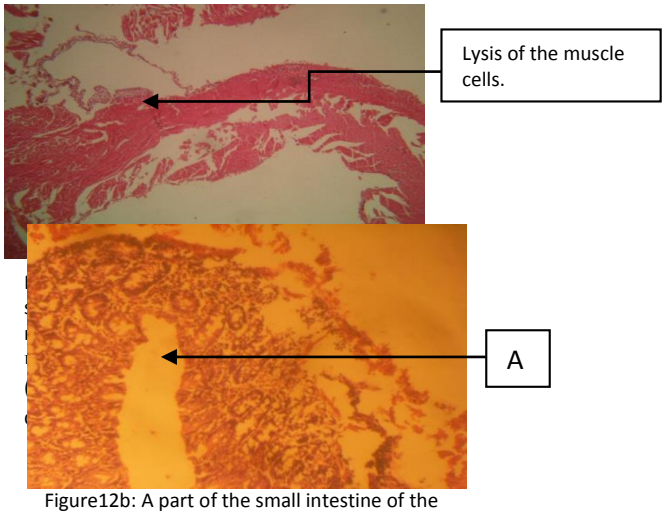

Figure12b: A part of the small intestine of the rat fed with *E. coli* showing degradation of the serosa (external part), moderate cellular vacuolation, dealignation and destruction of

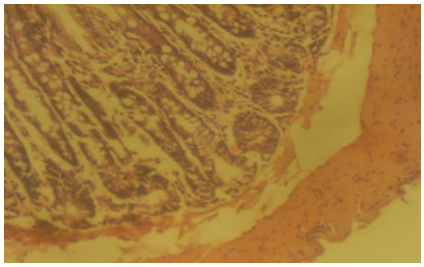

Figure 12a: A part of the small intestine of the rat used as control.

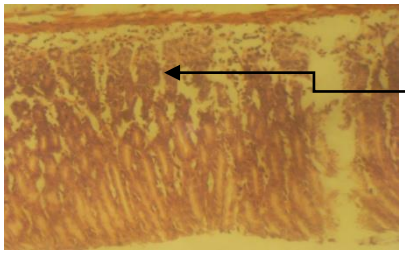

Plate 19: A part of the large intestine of rat fed with *Pseudomonas aeroginosa*showing severe egeneration of the mucosa, cellular

Table 1: Hematological tests analysis

S/N	SAMPLES	PCV (%)	X10^9 WBC	X10^{12} RBC	g/dl Hb	Neut. (%)	Lymph. (%)	Eosin. (%)
1	Control 1	42.00	6.50	4.62	13.80	20.00	80.00	_
2	Control 2	42.00	6.80	4.58	13.70	21.00	79.00	_
3	*P. aeruginosa 1*	40.00	10.60	4.41	12.80	26.00	74.00	_
4	*P. aeruginosa 2*	40.00	9.70	4.00	12.00	20.00	80.00	_
5	*K. pneumoniae.1*	41.00	7.60	4.50	13.10	10.00	90.00	_
6	*K. pneumoniae.2*	37.00	12.70	4.01	12.00	14.00	85.00	1.00
7	*Staph.aureus1*	39.00	12.00	3.98	12.30	28.00	71.00	1.00
8	*Staph. aureus2*	38.00	7.20	4.30	12.90	14.00	86.00	_
9	*E. coli 1*	44.00	11.60	5.20	14.50	12.00	88.00	_
10	*E. coli 2*	43.00	6.00	5.01	14.00	16.00	84.00	_

Keys:

Control 1 and Control 2: Rats serving as control

Pseudomonas aeruginosa 1&2: Rats fed with *Pseudomonas aeruginosa*

Klebsiella pneumoniae 1&2: Rats fed with *Klebsiella pneumoniae*

Staph.aureus 1&2: Rats fed with *Staphylococcus aureus*
E. coli 1&2: Rats fed with *Escherichia coli*

Table 1 shows an increase in white blood cell count (WBC) of rats that were fed with microbial isolates of food origin in relation to their respective PCV. An exception however is *E. coli* 2 group, whose WBC count was not in conformity with the range maintained by other infected rats and this could be as a result of variation in the immunity of the rats.

Discussion

The histopathological outcomes observed in this study are clear indications of the pathogenic capabilities of isolated microorganisms in albino rats. The liver of the control group shows normal tissue cell with no visible damage. The livers' cells could be intact as a result of no infection in the rats used as control. The cardiac muscles were also intact as normal cells; the cells of the muscle fibres were fully demonstrated. This could also be as a result of no infection in the rats.

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 by Fernandaz *et al.* [5]. The observation possibly indicates evidence of adherence factors and colonization of the intestine by a process described as pilation [6].

In rats fed with *E. coli*, it was observed that there was a wide separation in the sinusoid in the tissue of the liver compared with that of the control rats. This is referred to as Odematus. The endothelial cells of the central vein are not intact, there are aggregation of some lymphatic cells found in the liver tissue. There is an evidence of hemorrhage and lysis of cells. The separation in the sinusoid could be as a result of production of cytotoxic toxins by *E. coli* which have cytotoxic effects on the sinusoid in the tissues and the endothelial cells of the central veins thereby leading to their breaking down. The aggregation of some lymphatic cells could be as a result of immune

response to the production of toxins by *E. coli.* The hearts of rats fed with *E. coli* have cells of the muscles experiencing lysis and hemorrhage as the cell membrane of the veins were also broken and their nuclei which were supposed to be centrally placed are pushed to the periphery. This lysis could be as a result of the production of cytotoxic toxins by *E. coli* which destroyed the cells of cardiac muscles by causing lysis and hemorrhage of the cell membrane so that the nuclei are pushed to the periphery. Doyle *et al*. [7] reported similar findings on the advents of pathogenic *E. coli* on humans.

Large intestine of rat fed with *E. coli* shows severe destruction of the layers and degeneration of goblet cells. This is due to the invasive nature of this organism. Small intestine of the rat fed with *E. coli* shows degradation of the serosa (external part), moderate cellular vacuolation, dealignation and destruction of layers. This agrees with the work done by [8] which reports on the pathogenesis of *E. coli.* The entero-invasive *E. coli* (EIEC) attach specifically to the mucosa of the large intestine and invade the cells by being taken in by endocytosis. Inside the cell they lyse the endocytic vacuole and multiply and spread to adjacent cells causing tissue destruction.

In rat fed with *Escherichia coli*, it was observed that there was distortion of the renal capsule which is responsible for the filtration of plasma, there was distortion of both the Bowman`s capsule and the glomerulus. Also there was severe breakage of the basement membrane which hindered the Bowman`s capsule from resting on it, there was also fatty degradation seen in kidney of albino rats fed with *E. coli* compared with that of the control. This could be as a result of *E. coli* being able to produce cytotoxic toxin which has a cytotoxic effects on cells of the kidney or due to immune response to the production of toxin by *E. coli*. This agrees with the work of [9] who investigated the effect of *E. coli* infection on the histopathology of albino mice visceral organs. The spleen of rat fed with *E. coli* showed greater affinity to stain and mild hemorrhage, this could be as a result of toxins produced by *E. coli.* This also agrees with the work of [9] on effects of *E. coli* on the histopathology of albino mice visceral organs.

The liver tissues of rats fed with *Staphylococcus aureus* were normal, but there were some aggregations of lymphatic cells which may be as a result of infection with *Staphylococcus*. The cell

membranes of the veins were broken which may likely affect the endothelial lining of the cells. This may be as a result of the production of cytotoxins by *Staphylococcus aureus* which could lead to the destruction of cell membranes of the veins. The erythrocytes that are supposed to be in the sinusoids were not found at all. The reason for this could be as a result of the ability of the toxins produced by Staphylococcus to be haemolytic i.e. being able to lyse erythrocytes. Molecules produced by *Staphylococcus aureus* have been reported to produce enterotoxin and other toxin [10]. The hepatic cells were okay and the kupffer cells were also intact. The hearts of the rats fed with *Staphylococcus aureus* have cells which showed evidence of hemorrhage and little aggregation of lymphatic nodules.

The large lysis of the cells may be as a result of preparation. This is inferred due to the observation of little aggregation of lymphatic nodules. Kidney of rats fed with *Staphylococcus aureus* showed no effect on the cells of the kidney. This could be as a result of the immune system being able to fight the toxins produced by the organism or the organism itself thereby preventing it from reaching the kidney. Andrew *et al*. [11] reported similar effect produced by *Enterococcus faecalis* on the kidney of mice. The cells of spleen of rats fed with *S. aureus* showed mild multifocal fatty degradation and mild hemorrhage. The small intestine of rat fed with *Staphylococcus aureus* showed vacuolation of the secreting cells and hypertrophied plicae circulary/secreting cells. The large intestine of rat fed with *Staphylococcus aureus* shows vacuolation of secreting gland and dealignation of the basement (but not significant). Food-borne Staphylococci infections are associated the production of enterotoxin. The pathogenic effect of enterotoxin on the gastrointestinal tract results in intestinal lesions, suggesting a direct damaging effect on the intestinal epithelium [12]. Studies carried out by Bae *et al.* [13] found that α-hemolysin, a secreted protein that lyses mammalian host intestinal cells.

The livers of the rat fed with *Pseudomonas aeruginosa* have cells which show clear evidence of hemorrhage and a large aggregate of lymphoid tissue on the surface of the cells. This could be as a result of polymorphonuclear leukocytes (PMN) to engulf *Pseudomonas aeruginosa*. The hearts of the rats fed with *Pseudomonas aeruginosa* have cells which are okay and there was no significant

effect shown on the tissue. Rats` kidney fed with *Pseudomonas aeruginosa* have cells which showed mild hemorrhage and mild cellular degradation which was seen as patches. Phagocytosis by Polymorphonuclear leucocytes may have played a major role in the mild effect (hemorrhage) in the kidney of rat fed with *P. aeruginosa*. This could be because the PMN leucocyte is resistant to Pseudomonad infection. There is likelihood the immune system produces some antibody to somatic antigen and exotoxin produced by *Pseudomonas*. This agrees with the work of Bayo *et al.* [3]. The large and small intestines of rat fed with *Pseudomonas aeruginosa* shows severe degeneration of the mucosa, cellular destruction and lymphocyte infiltration, This cellular destruction could be due to the invasive nature of *P. aeruginosa* on deep tissues. Podolsky *et al.* [14] reported that in the gut, a naive lymphocyte that is activated in the Peyer's patches undergoes further differentiation in the local collecting lymph nodes (e.g., mesenteric lymph nodes) before returning to the blood as an immunoblast. The mucosa-derived immunoblast will then selectively re-enter the lamina propria of the gut rather than the peripheral lymph nodes to exert its effector functions. The spleen of rats fed with *P. aeruginosa* showed mild multifocal fatty degradation and mild hemorrhage. This also agrees with the work of Bayo *et al.* [3]. Rats fed with *Klebsiella pneumoniae* have liver cells which contain tissues in which hepatic drainage is highly affected. There is breakage of cell membranes. The sinusoids were largely separated and also some aggregations of lymphoid tissues were formed on the surface of the cells. A case of hemorrhage was also recorded. The cells of the heart of rats which were fed with *Klebsiella pneumoniae* were okay only for the hemorrhage that was noticed.

It was again observed that rats fed with *Klebsiella pneumoniae* showed mild cellular hemorrhage, there is blood stain in the glomerulus and there is vascular congestion, the vascular congestion could be caused by inflammation of the vascular tissue. The mild hemorrhage may be due to destruction of cortical radial vein. These could be as a result of pathogenic effects of *Klebsiella pneumoniae*. The cells of the spleen of rats fed with *Klebsiella pneumoniae* were okay except for the moderate vascular congestion noticed. The large intestine of rat fed with *Klebsiella pneumoniae* showing mild infiltration of lymphocytes. Tiny lymphatic vessels drain into a single larger vessel called a lacteal at the centre of the villi. Lymphocytes infiltration is indicative of the presence of an infection in the intestinal tract. Small intestine of rat fed with *Klebsiella pneumoniae*

showed moderate degeneration of the mucosa and vacuolation of goblet cell.

Conclusions

This study is indicative that the isolated foodborne pathogens have different effects on the internal organs of studied albino rats. Most of these foodborne pathogens and those that contaminate the food may cause damage of the various organs that sustain life.

References

[1] World Health Organization (2007). Foodborne Hazards in Basic Food Safety for Health Workers. World Health Statistics Quarterly, Volume 26.

[2] World Health Organization (WHO) (1997). Food safety and foodborne diseases. World Health Statistics *Quarterly*, Volume 50

[3] Bayo S, Petit JC, Sicard D (1980). Histopathological observation of experimental hematogenous infection with *Pseudomonas aeroginosa* in mice. *Pathol. Biol*. 28(4):241-246.

[4] Liu D (2004). *Listeria monocytogenes*: comparative interpretation of mouse virulence assay. *FEMS Microbiology Letters* 233:159–164.

[5] Fernandez H, Eller G, Paillacar J, Gajardo T, Riquelme A (1995). Toxigenic and invasive capacities: Possible pathogenic mechanisms in *Arcobacter cryaerophilus*. *Mem. Inst. Oswaldo. Cruz.* 90:633–634.

[6] Carbone M, Maugeri TL, Giannone M, Gugliandolo C, Midiri A, Fera MT (2003). Adherence of environmental *Arcobacter Arcobacter* and *Vibrio spp*. isolates to epithelial cells *in vitro*. *Food Microbiol.* 20: 611–616.

[7] Doyle MP, Zhao T, Meng J, Zhao S (1997). *Escherichia coli* O157:H7. *In*: Doyle MP, Beuchat LR, Montville TJ. (eds). *Food Microbiology - Fundamentals and Frontiers.* ASM, Washington DC, p.171-191.

[8] Gross RJ (1991). The pathogenesis of *Escherichia coli* diarrhea. *Rev Med Microbil* 2: 37- 44

[9] Abin B, Raja R, Kantha DA (2010). Effect of *Escherichia coli* infection on the histopathology of albino mice visceral organs. *International Journal of Engineering Science and Technology.* 2(3):259-263.

[10] Jablonski LM, Bohach GA (1997). Epidemiology of foodborne diseases. *In*: Doyle M.P, Beuchat LR, Montville TJ (eds). *Food Microbiology - Fundamentals and Frontiers.* ASM, Washington DC, p. 353-375.

[11] Andrew LK, Steven M.M, William L, Ericka H, Micheal GC, Scott JH (2005). *Enterococcus faecalis* tropism for the kidneys in the urinary tract of C57BL/6J mice. *Infection and immunity*. 73(4):2461-2468.

[12] Campbell WN, Fitzpatrick M, Ding X, Jett M, Gemski P, Goldblum SE (1997). SEB is cytotic and alters EC barrier function through protein tyrosine phosphorylation invitro. *Am.J. Physiology*. 273: L31-39.

[13] Bae T, Banger AK, Wallace A, Glass EM, Aslund F, Schneewind O, Missiakas DM (2004). *Staphylococcus aureus* virulence genes identified by *Bursa aurealis* mutagenesis and nematode killing. *Proc. Natl. Acad. Sci*. USA *101*: 12312–12317.

[14] Podolsky DK, Lynch-Devaney K, Stow JL, Oates P, Murgue B, DeBeaumont M, Sands BE, Mahida YR (1993). Identification of human intestinal trefoil factor. Goblet cell- specific expression of a peptide targeted for apical secretion. *The Journal of Biological Chemistry*, 268: 6694- 670

Authors Column

Dr. I. I. Anibijuwon is a lecturer at the Department of Microbiology, University of Ilorin, Nigeria. He is specialized in Medical microbiology and Public health. His research interests are antimicrobial resistance, infectious diseases, and antimicrobial activities of natural products. He is a member of the Nigerian Society of Microbiology and American Society of Microbiology. Dr. Ibikunle Anibijuwon has more than 8 years teaching and research experience in the field of Microbiology.