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Pathogenicity of *Salmonella enteritidis* Phage Type 1 Isolate of Malaysia in 21 Day Old Specific-Pathogen Free Chickens

S. Ahmad, M. Hair-Bejo, Z. Zunita and S. Khairani-Bejo
Faculty of Veterinary Medicine, Universiti Putra Malaysia,
43400 UPM Serdang, Selangor, Malaysia

Abstract: *Salmonella enteritidis* (SE) has always been related to subclinical infection in the chickens infected after 2 weeks of hatching. However, few pathogenic phage types were proven for their ability to manifest systemic infection and cause the organism to be shed into the surrounding environment. It was the objective of the study to determine the pathogenicity of SE Phage Type (PT) 1 in Specific-Pathogen-Free (SPF) chickens. About 93, 21 day old SPF chickens where divided into 3 groups namely the Control, SE and Mortality groups. The chickens were raised separately in caging system and given free access to antibiotic-free ration and water. The SE and Mortality groups were inoculated orally (1.0 mL) with SE PT 1 (1×10^8 cfu mL^{-1}). The chickens in the SE and Control groups were sacrificed at various intervals throughout the trial. Samples were collected for bacterial isolation and histological examination. The mortality percentage of the chickens in the Mortality group was recorded. The study showed that no mortality was recorded throughout the trial in the mortality as well as the SE group. Body weight was lower in the SE group when compared to the Control group throughout the trial except at days 2, 3 and 5 post inoculation (pi) reaching its peak at day 14 pi when the SE group body weight was 26% lower than the controls. Clinical signs observed in the SE and Mortality group were represented by diarrhoea, inappetance, ruffled feather and stunted chickens while no abnormal clinical signs where recorded in the Control group. Grossly mild airsacculitis, mild peritonitis and hepatic congestion where recorded in the SE group at day 2 pi until day 5 pi while no gross lesions where recorded in the Control group. SE was first isolated in the caecum (66%) at 12 h pi. At day 1 pi SE was isolated from the caecum and spleen (33%) whilst at day 2, SE was isolated from the caecum (100%) and caecal tonsil (66%). No SE was isolated from the cloacal swabs throughout the trial. The villi height was generally lower in the SE group when compared to the Controls, however it was significantly lower ($p<0.05$) in the duodenum at 12 h, days 1, 3, 5, 10, 14 and 21 pi; in the jejunum at 6 h, days 2, 14 and 21 pi while in the ileum at days 1, 3 and 5 pi. The crypts depth measurement was fluctuating however it ended up by being higher in the SE group, nevertheless it was significantly lower ($p<0.05$) in the SE group when compared to the Control group in the duodenum at 6 h and day 14 pi in the jejunum at day 10 pi; in the ileum at 12 h pi. Histopathological changes recorded included hepatitis, congestion and focal areas of necrosis; splenitis, congestion and oedema in the adenoid sheathed arteries; congestion and areas of necrosis in the lymphoi follicles of the bursa of Fabricius; enteritis, congestion and sloughing of necrotic enterocytes in the intestinal villi with presence of bacterial clusters in the villi surface and intestinal lumen. SE rods present in the caecal tonsils were seen to be engulfed by macrophages at days 1 and 2 pi, necrosis of the enterocytes on the villi surface and infiltration of the bacteria was recorded at day 2 pi while at days 5 pi the bacteria multiplication were seen and often located upon the M-like M cells however, no actual engulfment was recorded.

Key words: *Salmonella enteritidis*, phage type 1, pathogenicity, adult SPF chickens, bacteria, Malaysia

INTRODUCTION

Paratyphoid infection has long been known as a major cause for food poisoning outbreaks worldwide by consumption of contaminated products (Saif, 2003). In the last decade *Salmonella enteritidis* (SE) outbreaks has elevated in its incidence rate to become the major source for food poisoning cases caused by paratyphoid *Salmonella* infection around the world (Le Bacq *et al*., 1994). Chickens are more susceptible to the SE infection at younger ages due to the lack of normal microflora and susceptibility to infection decrease dramatically after

Corresponding Author: M. Hair-Bejo, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

2 weeks of hatching (Desmidt *et al*., 1997). Adult chickens tend to act as subclinical carrier to the paratyphoid infection (Kinde *et al*., 2000). SE has many Phage Types (PT's) that varied in their pathogenicity and prevalence around the globe. SEPT 4 has been the major isolate in poultry and human SE infection outbreaks worldwide (Kinde *et al*., 2000). Other PT's such as SEPT 6, 8 and 13a have prevailed over the years in different regions (Gast and Beard, 1990; Barrow and Lovell, 1991; Kinde *et al*., 2000). However, their isolation rates remained lower than that of SE PT 4. SE PT 8 and 13a are the major isolates in the United States and Canada while PT4 was predominantly isolated in the United Kingdom. There has been an increased in the isolation percentage of SE PT 1 in Europe and Asia and other countries (Sakai and Chalermchaikit, 1996). A few studies indicated the ability of pathogenic SEPT's to induce a clinical infection and reduce body weight in the infected chickens and persist in the infected birds for long periods (Davies *et al*., 1997). The infection usually exaggerates in the immune compromised or stressed chicks by mixed infection or bad management (Soliman *et al*., 2009). The contamination of the adult birds will certainly form a threat for the human consumers due to contamination of poultry and their products by the pathogens. It was the objective of the study to determine the pathogenicity of SEPT 1 in 21 day old Specific-Pathogen-Free (SPF) chickens.

MATERIALS AND METHODS

Bacterial isolates: Samples of liver from commercial broiler farm in Melaka, Malaysia was collected and identified as SE using a method of Van Der Zee (1994). Briefly, the samples were collected in Rappaport-Vassilidase (RV) broth at 37°C for 24 h, cultured on Xylose Lysin Deoxycholate (XLD) agar and Brilliant Green (BG) agar at 37°C for 24 h. Positive cultures where then cultured on Triple Sugar Iron (TSI) and Urease biochemical test. Positive cultures from the biochemical test where then identified serologically by Salmonella poly-O antisera using the Slide Agglutination Test (SAT).

Phage typing: Positive SE colonies where characterised by phage typing at the Laboratory of Enteric Pathogens Center for Infections Institute, 61 Collindale Avenue, London, United Kingdom by the method described by Ward *et al*. (1987). Briefly, a loopful of frozen nutrient broth was cultured on nutrient agar for 24 h at 37°C. The positive colonies were transferred to BA cultured and incubated for 24 h at 37°C. The colonies from BA culture were inoculated into 3 mL of phage broth for 2 h with vigorous shaking. The phage broth was then poured on phage agar plate with the removal of excess broth. After that bacteriophages were applied and left to dry in room temperature for an overnight incubation at 37°C the phage lysis pattern of each culture was then evaluated according to Ward *et al*. (1987) scheme. Resulted phage type was SEPT 1.

Inoculums preparation: SE colonies where cultured on nutrient agar for 24 h at 37°C. The positive cultures were then cultured on BA for 24 h at 37°C. The positive cultures were then harvested and placed in normal saline solution and then measured for the inoculums concentration by using the McFarland standard to prepare the inoculum in a concentration of 1×10^8 colony forming unit (cfu) per mL.

Specific-pathogen-free chickens: Specific-Pathogen-Free (SPF) embryonated chicken eggs where obtained from the Veterinary Research Institute (VRI), Ipoh, Malaysia. The eggs where incubated (Black Chick, Siam Incubator System, Thailand) in Biologic Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia and upon hatching they were transferred into separate cages and granted free access to antibiotic-free ration and water supply. The cages where supported by artificial lighting and ventilation system.

Experimental design: About 21 day old chicks were divided into 3 groups namely SE, Control and Mortality groups. The chicks in SE and Mortality groups were orally inoculated with 0.1 mL of the SEPT 1 inoculum in a concentration of 1×10^8 cfu mL^{-1} while the Control group was not inoculated. About 3 chicks were humanly sacrificed by cervical dislocation prior to SE inoculation for bacteriological isolation and body weight measurement and from the SE and Control groups at every sampling dates at 6 and 12 h post inoculation (pi), 1, 2, 3, 5, 7, 10, 14 and 21 days (d) pi. Body weight was recorded from the chicks in SE and Control groups at the sampling dates. Clinical signs were observed at least twice daily throughout the trial. On necropsy gross lesions were observed. Samples of liver, spleen, blood, contents of the middle part of small intestine, caecum, caecal tonsils and cloacal swabs were collected in RV broth and incubated for 24 h at 37°C for SE isolation. Samples including liver, spleen, duodenum, jejunum, ileum and caecum were collected and fixed in 10% buffered formalin for histological examination. Caecal tonsils were also collected and fixed in 2.5% glutaraldehyde cacodylate buffer, pH 7.2 at 4°C for ultrastructural examination using Scanning Electronic Microscopy (SEM).

Bacterial isolation: Sample of liver, spleen, blood, contents of small intestine, caecum, caecal tonsils and cloacal sawbs where collected in RV broth at 37°C for 24 h, then cultured on XLD agar and BG agar at 37°C for 24 h. Positive colonies were then cultured on TSI and Urease biochemical tests. Positive colonies from the biochemical test were then identified serologically using the SAT (Van Der Zee, 1994).

Histopathology: Samples of liver, spleen, bursa of Fabricius, duodenum, jejunum, ileum and caecum were collected and fixed in 10% buffered formalin for 48 h. Samples where processed and stained with Hematoxylin and Eosin (HE) (Bancroft *et al*., 1996). Briefly, the samples were trimmed into the required orientation and processed for dehydration in series of alcohol, cleaned with xylene using an automated tissue processor (Leica ASP 300, Germany). Samples were then embedded in paraffin (Leica EG 1160, Germany), trimmed and sectioned at 4 μm on microtome (Leica RM 2155, Germany). The paraffin sections where then mounted on glass slides (Leica Hi 1220). The slides were then stained using Hematoxylin and Eosin (HE) (Bancroft *et al*., 1996). Histopathological changes were recorded by examining the samples under 10, 20, 40 and 100x objectives using the light microscope (Leica, Germany). For morphometric analysis, intestinal segments from duodenum, jejunum and ileum samples were examined under 10 and 20x objectives for the villus height and crypts depth measurement, respectively. Villus height was measured from the tip of the villi to the villus crypt junction, whilst crypt depth was measured from the base of the crypt to the crypt villi junction.

Scanning electron microscopy: Caecal tonsils samples were taken from chicks sacrificed at 6 and 12 h, days 2, 5, 7 and 21 pi and fixed in 2.5% glutaraldehyde cacodylate buffer for 12 h and processed for viewing by the SEM (Watson *et al*., 1995). Briefly, samples where fixed in glutaraldehyde buffer for 12 h then washed with 0.1 M sodium cacodylate buffer for 3 changes of 10 min each. Samples were then fixed with osmium terta-oxide for 2 h and washed with the 0.1 M sodium cacodylate buffer for 3 changes of 10 min each. Then samples were dehydrated with series of acetone and run for the critical point drying, mounted on a double sided tape or colloidal silver. It was then coated with gold sputter or coater samples and viewed under SEM.

RESULTS AND DISCUSSION

Body weight: The body weight in the SE group increased continuously starting day 7 pi and thereafter. When compared to the Control, SE group was lower throughout the trial, except at days 2, 3, 5 pi, however the significant decreased ($p<0.05$) was only recorded at days 7 and 14 pi (Table 1).

Table 1: The body weight of SPF chickens in the control and SE groups throughout the trial

	Body weight (mean+SD, g)*	
Date (pi)	Control	SE
0 h	$164\pm3^{a,c}$	$164\pm3^{a,c}$
6 h	$205.5\pm8.2^{a,d}$	$204.8\pm4.4^{a,d}$
12 h	$214\pm7^{a,d}$	$196.1\pm24.2^{a,d}$
Day 1	$228.8\pm31.3^{a,d}$	$217.3\pm34^{a,d}$
Day 2	$231.3\pm4.7^{a,d}$	$288.1\pm0.8^{a,d}$
Day 3	$260.9\pm9.1^{a,e}$	$262.6\pm40.1^{a,d}$
Day 5	$249.3\pm11.5^{a,f}$	$281.8\pm10.7^{b,d}$
Day 7	$332.2\pm15.9^{a,g}$	$261.3\pm13.5^{b,e}$
Day 10	$334.4\pm23.6^{a,g}$	$303.4\pm7.3^{a,f}$
Day 14	$469.3\pm52.9^{a,h}$	$350.3\pm22.9^{b,g}$
Day 21	$501.6\pm27.5^{a,h}$	$451\pm44.1^{a,h}$

*The mean with b in the superscript in the SE group means it vary significantly with the other mean in the same row while the mean with different letter (c-h) in the superscript means it vary significantly with the previous mean within the same column; level of significance was accepted at ($p<0.05$)

Clinical signs: No abnormal clinical signs where observed in the Control group throughout the trial. However, diarrhoea was recorded in 100% of the examined chicks starting day 2 pi until day 7 pi. Inappetance was recorded starting day 3 pi and thereafter while ruffled feathers and stunting where recorded stating day 5 pi and thereafter. Diarrhoea was observed in the Mortality group (100%) starting day 2 pi and it lasted until day 10 pi. At day 3 pi and thereafter inappetance was observed. Ruffled feathers and stunting was observed starting day 5 pi and thereafter.

Gross lesions: No gross lesions where recorded in the Control group throughout the trial. However, in the SE group mild airsacculitis and mild peritonitis where recorded at days 2 pi (66%), 3 pi and 5 pi (100%). Liver congestion was recorded at days 3 and 5 pi (33%). No gross lesions where recorded thereafter (Fig. 1).

Bacteriological isolation: No SE was isolated in the Control group throughout the trial. In the SE group, SE was isolated from liver only at day 3 pi (33%); from spleen at days 1 and 3 pi (33%); from blood samples at day 3 pi (33%); from contents of small intestine at day 3 pi (33%); from caecum contents at 12 h pi (66%), day 1 and 3 pi (33%) and day 2 pi (100%); from caecal tonsils at day 2 pi (66%); no SE was isolated from cloacal swabs throughout the trial.

Villi length

Duodenum: The villi height in the SE group was fluctuating throughout the trail. When compared to the Control group the villi height in the SE group was lower

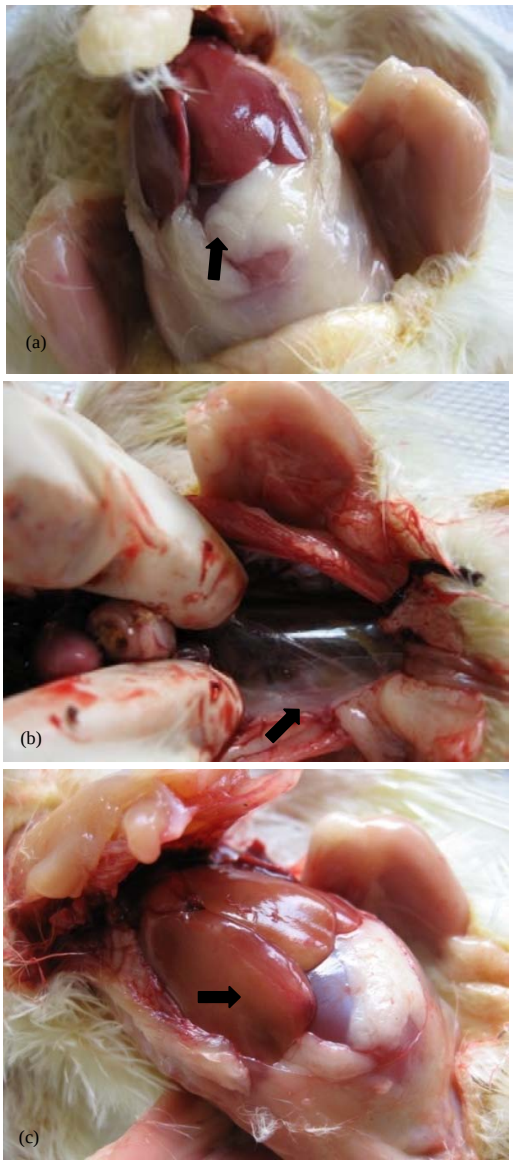

Fig. 1: The gross lesions of SPF chickens in the SE group. (a) Mild peritonitis; (b) Mild airsacculitis at day 5 pi and (c) Mild hepati ccongestion at day 5 pi

throughout the trial however, significant decreased ($p<0.05$) was recorded at 12 h, days 1, 3, 5, 10, 14 and 21 pi (Table 2).

Jejunum: The villi height measurement in the SE group was fluctuating, however it increased continuously starting day 14 pi and thereafter. When compared to the Control group the SE group villi height measured lower throughout the trial except at day 1 pi however, the decreased was significant ($p<0.05$) at 6 h, days 2, 14 and 21 pi (Table 3).

Table 2: The duodenal villus height of SPF chickens in the control and SE groups throughout the trial

	Villus height (mean+SD, μm)*	
Date (pi)	Control	SE
0 h	1160±87^{a,c}	1160±87^{a,c}
6 h	1349±198^{a,c}	1082±155^{a,c}
12 h	1197±82^{a,c}	1000±47^{b,c}
Day 1	1401±16^{a,d}	899±146^{b,c}
Day 2	1246±171^{a,d}	974±172^{a,c}
Day 3	1168±194^{a,d}	771±169^{b,c}
Day 5	1299±27^{a,d}	934±85^{b,c}
Day 7	1274±165^{a,d}	1175±82^{a,d}
Day 10	1587±48^{a,e}	1279±42^{b,d}
Day 14	1733±72^{a,f}	1230±62^{b,d}
Day 21	1885±55^{a,g}	1200±22^{b,d}

*The mean with b in the superscript in the SE group means it vary significantly with the other mean in the same row while the mean with different letter (c-g) in the superscript means it vary significantly with the previous mean within the same column; level of significance was accepted at ($p<0.05$)

Table 3: The jejunal villus height of SPF chickens in the control and SE groups throughout the trial

	Villus height (mean+SD, μm)*	
Date (pi)	Control	SE
0 h	577±97^{a,c}	577±97^{a,c}
6 h	798±63^{a,d}	645±70^{b,c}
12 h	806±95^{a,d}	705±72^{a,c}
Day 1	730±42^{a,d}	786±35^{a,c}
Day 2	874±56^{a,e}	661±78^{b,d}
Day 3	842±110^{a,e}	697±69^{a,d}
Day 5	1012±191^{a,e}	820±8^{a,e}
Day 7	1077±54^{a,e}	1040±51^{a,f}
Day 10	1116±126^{a,e}	1017±76^{a,f}
Day 14	1207±59^{a,e}	911±74^{b,f}
Day 21	1263±8^{a,e}	1165±78^{b,g}

*The mean with b in the superscript in the SE group means it vary significantly with the other mean in the same row while the mean with different letter (c-g) in the superscript means it vary significantly with the previous mean within the same column; level of significance was accepted at ($p<0.05$)

Ileum: The villi height in the SE group was fluctuating however, it continuously increased starting day 10 pi and thereafter. When compared to the Control group the villi height in the SE measured lower than the Controls starting 12 h pi and thereafter except at days 7 and 10 pi however, significant decreased ($p<0.05$) was recorded at days 1, 3 and 5 pi (Table 4).

Crypts depth

Duodenum: The crypts depth measurement in the SE group was fluctuating, however it increased continuously starting day 7 pi and thereafter. The crypts depth in the SE group was lower throughout the trial when compared to the Control group, except days 5 and 21 pi, however it was significantly lower ($p<0.0.5$) at 6 h and day 14 pi (Table 5).

Jejunum: The crypts depth measurement in the SE group was fluctuating throughout the trial. The SE group crypts depth was lower when compared to the Controls at 6 and 12 h, days 1, 2 and 10 pi however, it was significantly lower ($p<0.05$) at day 10 pi only (Table 6).

Table 4: The ileum villus height of SPF chickens in the control and SE groups throughout the trial

	Villus height (mean+SD, μm)*	
Date (pi)	Control	SE
0 h	537±39^{a,c}	537±39^{a,c}
6 h	604±22^{a,d}	645±74^{a,d}
12 h	703±83^{a,d}	670±51^{a,d}
Day 1	633±5^{a,d}	565±52^{b,e}
Day 2	704±241^{a,d}	653±111^{a,e}
Day 3	720±41^{a,d}	594±26^{b,e}
Day 5	753±30^{a,d}	604±32^{b,e}
Day 7	797±65^{a,d}	882±98^{a,f}
Day 10	866±108^{a,d}	873±126^{a,f}
Day 14	1112±49^{a,e}	1052±45^{a,g}
Day 21	1108±47^{a,e}	1063±73^{a,g}

*The mean with b in the superscript in the SE group means it vary significantly with the other mean in the same row while the mean with different letter (c-g) in the superscript means it vary significantly with the previous mean within the same column; level of significance was accepted at ($p<0.05$)

Table 5: The duodenum crypts depth of SPF chickens in the control and SE groups throughout the trial

	Crypts depth (mean+SD, μm)*	
Date (pi)	Control	SE
0 h	68±10^{a,c}	68±10^{a,c}
6 h	70±9^{a,c}	58±2^{b,c}
12 h	61±14^{a,c}	56±2^{a,c}
Day 1	70±12^{a,c}	65±1^{a,d}
Day 2	67±2^{a,c}	63±8^{a,d}
Day 3	67±4^{a,c}	57±6^{a,d}
Day 5	60±11^{a,c}	68±8^{a,d}
Day 7	68±2^{a,c}	62±4^{a,d}
Day 10	98±27^{a,c}	71±5^{a,e}
Day 14	86±5^{a,c}	75±4^{b,e}
Day 21	83±7^{a,c}	84±4^{a,f}

*The mean with b in the superscript in the SE group means it vary significantly with the other mean in the same row while the mean with different letter (c-f) in the superscript means it vary significantly with the previous mean within the same column; level of significance was accepted at ($p<0.05$)

Table 6: The jejunal crypts depth of SPF chickens in the control and SE groups throughout the trial

	Crypts depth (mean+SD, μm)*	
Date (pi)	Control	SE
0 h	60±5^{a,c}	60±5^{a,c}
6 h	61±5^{a,c}	63±3^{a,c}
12 h	55±8^{a,c}	58±1^{a,d}
Day 1	59±4^{a,c}	58±6^{a,d}
Day 2	62±6^{a,c}	61±9^{a,d}
Day 3	59±8^{a,c}	62±3^{a,d}
Day 5	50±3^{a,c}	64±4^{b,d}
Day 7	68±4^{a,d}	75±5^{a,e}
Day 10	86±4^{a,e}	67±6^{b,f}
Day 14	72±4^{a,f}	77±8^{a,f}
Day 21	72±3^{a,f}	76±4^{a,f}

*The mean with b in the superscript in the SE group means it vary significantly with the other mean in the same row while the mean with different letter (c-f) in the superscript means it vary significantly with the previous mean within the same column; level of significance was accepted at ($p<0.05$)

Ileum: Crypts depth in the SE groups was fluctuating as well throughout the trial. The crypts depth in the SE group was lower when compared to the Control group at 6 and 12 h, days 1, 2 and 10 pi however, significance ($p<0.05$) was detected at 12 h pi only (Table 7).

Table 7: The ileum crypts depth of SPF chickens in the control and SE groups throughout the trial

	Crypts depth (mean+SD, μm)*	
Date (pi)	Control	SE
0 h	51±3^{a,c}	51±3^{a,c}
6 h	57±2^{a,d}	55±2^{a,c}
12 h	60±1^{a,d}	48±2^{b,d}
Day 1	52±2^{a,e}	51±1^{a,d}
Day 2	59±5^{a,f}	56±1^{a,e}
Day 3	54±6^{a,f}	59±5^{a,e}
Day 5	53±6^{a,f}	55±7^{a,e}
Day 7	63±5^{a,g}	64±6^{a,e}
Day 10	71±3^{a,g}	62±7^{a,e}
Day 14	74±3^{a,g}	75±2^{a,e}
Day 21	70±9^{a,g}	75±4^{a,f}

*The mean with b in the superscript in the SE group means it vary significantly with the other mean in the same row while the mean with different letter (c-g) in the superscript means it vary significantly with the previous mean within the same column; level of significance was accepted at ($p<0.05$)

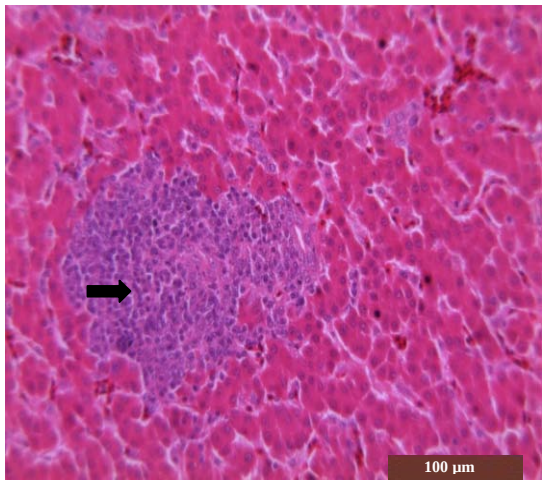

Fig. 2: The histopathological changes in the liver of SPF chickens in the SE group. Hepatitis and areas of necrosis at day 5 pi. HE, Bar = 100 μm

Histopathological changes: No histopathological changes were detected in the Control group throughout the trial. However, mild hepatitis was recorded in the SE group starting 6 h pi until day 1 pi while moderate hepatitis and congestion where recorded starting day 2 pi until day 5 pi. Mild hepatitis, congestion and areas of focal necrosis were observed starting day 5 pi and thereafter (Fig. 2). Mild splenitis was recorded in the SE group starting 6 h pi until day 1 pi. Mild splenitis and congestion was recorded starting day 2 pi and thereafter. Areas of hyperplastic islands, increased hetrophilic infiltration and oedema surrounding the adenoid sheathed arteries were observed at day 7 pi and thereafter (Fig. 3). Mild inflammation of the bursa of Fabricius was recorded in the SE group starting 6 h pi until day 2 pi. Mild inflammation, congestion, degeneration and necrosis in the lymphoid follicles were observed starting day 3 pi and thereafter (Fig. 4). Mild

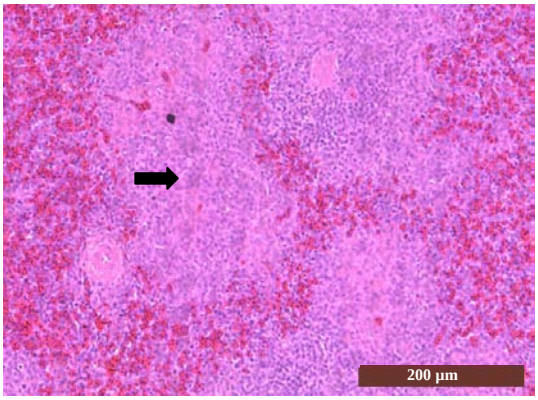

Fig. 3: The histopathological changes in the spleen of SPF chickens in the SE group. Congestion, increased cellularity and hyperplastic cells at day 7 pi. HE, Bar = 200 µm

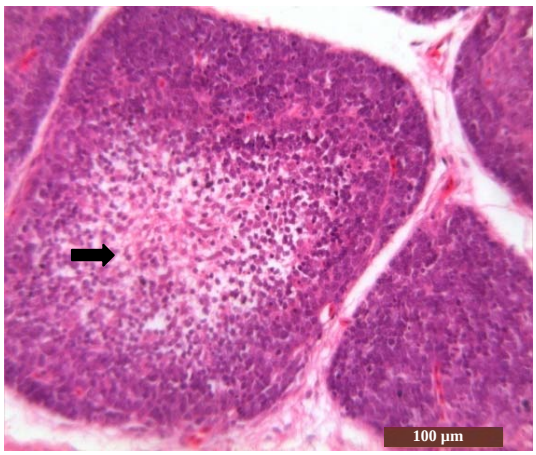

Fig. 4: The histopathological changes in the bursa of Fabricius of SPF chickens in the SE group. Degeneration and necrosis of lymphoid cells at day 2 pi. HE, Bar = 100 µm

enteritis was observed in duodenum, jejunum, ileum and caecum in the SE group at 6 and 12 h pi. Mild enteritis, congestion and sloughing of the necrotised enterocytes into the villi lumen was recorded in the intestine and bacterial clusters where seen passing to the intestinal villi through sloughed epithelium stating day 5 and thereafter (Fig. 5-8).

Scanning electron microscopy: SE found to be present in the caecal tonsils interepithelial junctions and were seen to be engulfed by macrophages in the SE group at day 1 pi (Fig. 9). At day 2 pi, engulfment of the SE by the macrophages and necrosis of the enterocytes in the

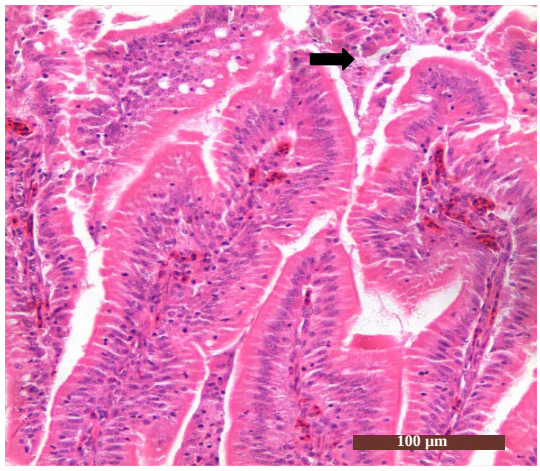

Fig. 5: The histopathological changes in the duodenum of SPF chickens in the SE group. Congestion of the duodenal villi and sloughing of the necrotic enterocytes with bacterial custers in the intestinal lumen. HE, Bar = 100 µm

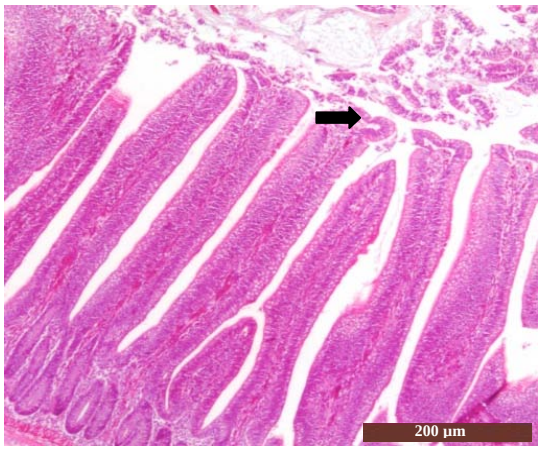

Fig. 6: The histopathological changes in the jejunum of SPF chickens in the SE group. Sloughing of the necrotic enterocytes into the intestinal lumen at day 7 pi. HE, Bar = 200 µm

caecal tonsils villi surface was recorded with infiltration of the bacteria through these areas (Fig. 10). At day 5 pi, bacterial multiplication where seen and often they were located by the M-like M cells however no actual engulfment was recorded.

The paratyphoid infection normally cause a subclinical infection in adult chickens infected after 2 weeks of hatching (Girard-Santosuosso *et al*., 1998), however few experimental studies showed the susceptibility of chickens to be infected using

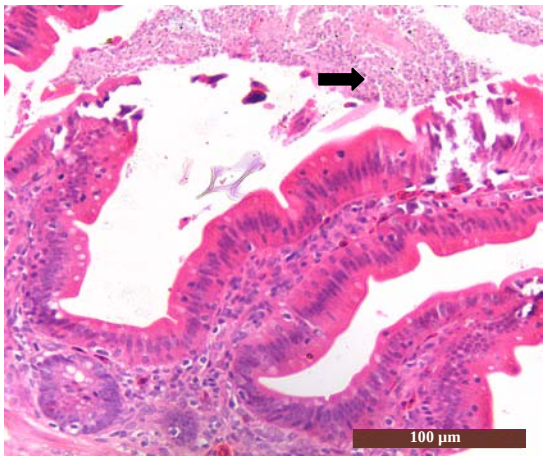

Fig. 7: The histopathological changes in the ileum of SPF chickens in the SE group. Necrosis of the enterocytes and bacterial clusters in the intestinal lumen at day 10 pi. HE, Bar = 100 µm

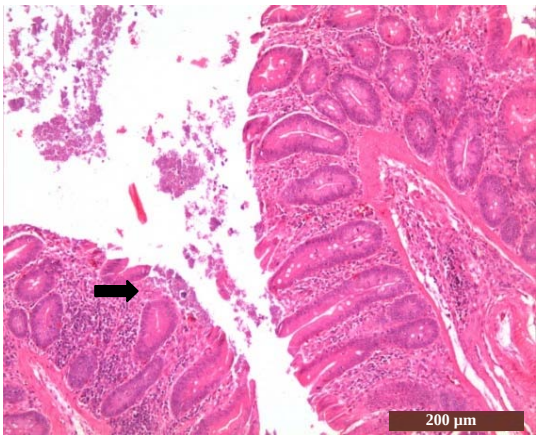

Fig. 8: The histopathological changes in the caecum of SPF chickens in the SE group. Necrosis of the enterocytes and bacterial clusters infiltration into the intestinal villi at day 7 pi. HE, Bar = 200 µm

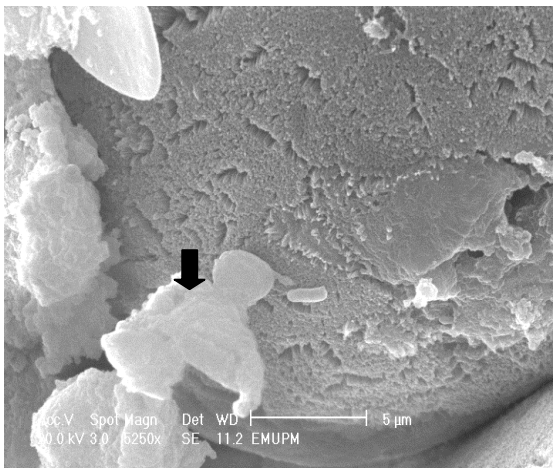

Fig. 9: The ultrastructural changes in the caecal tonsil of SPF chickens in the SE group. Bacterial engulfment by the macrophages at day 1 pi. SEM, Bar = 5 µm

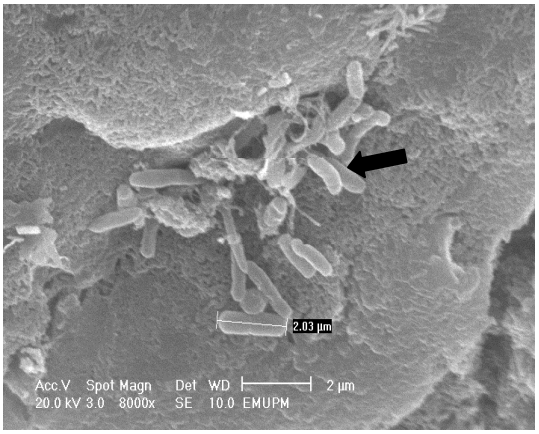

Fig. 10: The ultrastructural changes in the caecal tonsils of SPF chickens in the SE group. Bacterial infiltration through the enterocyte at day 2 pi. SEM, Bar = 2 µm

pathogenic phage types and high inoculums concentration (Holt, 1993). The infection of adult chickens defiantly raise the alarm for environmental contamination and elevate the risk of food poisoning outbreaks caused by consumption of poultry or poultry products polluted by the these pathogens (Dhillon *et al*., 1999). In the present study the oral inoculation of 21 day old SPF chickens resulted in mild pathogenic infection. However, the body weight in the SE group was lower throughout the trial except for days 2, 3 and 5 pi reaching its peak at day 14 pi when SE measured 26% lower than the controls.

Clinical signs observed in the infected chickens included diarrhoea, inappetance, ruffled feathers and stunting of the chickens in the period starting day 2 pi and thereafter while no abnormal clinical signs were seen in the Control group. Although, the profuse watery diarrhea started at day 2 pi in all the chickens examined and lasted until day 7 pi in the SE group however the effect on weight loss didn't show up until day 7 pi while earlier studies showed a significant change in body weight throughout the trial in adult infected chickens (Eigaard *et al*., 2006). The gross lesions observed between day 2 pi to day 5 pi included mild airsacculitis, mild peritonitis and liver congestion.

The intensity of clinical and gross findings coordinated with bacteriological isolation when 33% of liver, spleen, blood, jejunum and caecum where positive for SE at day 3 pi. Nevertheless the first isolation recorded was from the caecum at 12 h pi. The caecum was the most consistent site for SE isolation, SE was isolated from 66% at 12 h pi; 33% at days 1 and 3; 100% at day 2pi. First isolation of the SE from visceral organs was recorded in the spleen at day 1 pi (33%) which marks the primary bactaeremia while day 3 pi might be the secondary bactaeremia mark as the pathogen can be isolated in bigger percentage from different sites in the chicken's body. This delay might indicate the enhancement of immune response of the adult chickens. However, interestingly observed but poorly understood no SE was isolated from the cloacal swabs throughout the trial, this might be related to the low concentration the organism was shed in which might cause the failure of the conventional methods to detect its presence. Nevertheless previous study recorded the persistence of SE in infected chickens for long periods after inoculation (Reiber *et al*., 1995; Shivaprasad *et al*., 1990).

The ration absorption is majorly occurs in the intestinal segment of the Gastrointestinal Tract (GIT) and the morphology of the villi and crypts depth will be a major factor in evaluating the eventual ration conversion rate and the ultimate chickens body weight (Uni *et al*., 1999). The intestinal villi in duodenum, jejunum and ileum where generally affected in the SE group and where lower when compared to the control. Crypts depth where affected as well however, the impact was lower than the one the villi had. Nevertheless, the crypts depth ended up by being higher in the SE group in the end of the trial at day 21 pi in the jejunum and ileum. This lower impact in the intestinal crypt might refer to the presence of the segmented filamentous bacteria which an autochthonous bacterium is colonizing the ileum of many young animals by attachment to intestinal epithelial cells. These non pathogenic bacteria strongly stimulate the mucosal immune system by elevating immunoglobulin A-secreting cells (Klaasen *et al*., 1993) and have a possible protective role against SE infection (Garland *et al*., 1982).

Heterophilic infiltration in the liver, spleen, bursa of Fabricius, duodenum, jejunum, ileum and caecum indicated the inflammatory response of the infected tissues to the SE presence. However, progressive development of the histological changes in liver represented by focal necrosis, in spleen by hyperplastic islands and oedema of the adenoid lymphatic sheathed arteries, in the bursa by the areas of necrosis in the lymphoid follicles and in the intestine by the necrosis and sloughing of the enterocytes and infiltration of the bacteria indicated the continuous pathogenic effect of the bacteria and presence inside the body although no positive isolation was detected further than day 3 pi.

This was further demonstrated by detecting the bacteria under the SEM at day 5 pi in the caecal tonsils where multiplication of the bacteria where seen, however at earlier times at days 1 and 2 pi the intake of the organism was seen to take place by the engulfment of the bacteria by the macrophages and the infiltration of the bacteria through sloughed enterocytes on the villi surface in the caecal tonsils at day 2 pi. This might suggest that after day 2 pi the bacterial concentration in the caecal tonsils was lower than day 1 and 2 pi either by engulfment or penetration of the bacteria into the lamina propria, nevertheless a potential increase might occur in the stressed or immune suppressed chicks.

CONCLUSION

It was concluded that the SE PT 1 isolates of Malaysia is mild pathogenic to 21 day old SPF chickens. SE can successfully cause significant weight loss ($p<0.05$) and systemic infection represented by bacterial isolation from visceral organs and the impact caused grossly and histologically on the examined organs which might be exaggerated in stressed or immune suppressed chickens. Although, the organism was not detected by the conventional methods used for isolation, however its presence was detected by the SEM examination and histopathological changes caused which strongly suggest the improvement of screening methods applied for SE detection.

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