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PATHOGENICITY OF *SALMONELLA ENTERITIDIS* PHAGE TYPES 3A AND 35 AFTER EXPERIMENTAL INFECTION OF WHITE LEG HORN CHICKS

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ABSTRACT

Out of 155 newly hatched SPF White Leghorn chicks, five chicks were randomly separated to confirm the SPF status of the chicks before inoculation. The remaining 150 chicks were divided into six groups. The three sacrificed groups (A, B and C) of 30 chicks each and their respective three mortality groups (MA, MB and MC) of 20 chicks each. The chicks in groups A and MA, and in groups B and MB were challenged orally with 0.1mL containing 10^7 cfu of SE phage type 3A (UPM-0541) and SE phage type 35 (UPM-0525), respectively. The un-inoculated groups C and MC served as negative controls. Pathogenicity of *Salmonella enterica* serovar enteritidis (S.Enteritidis) phage types (PTs) 3A and 35 infections was determined through inoculation orally with (0.1mL/chick) 10^7 colony forming units (cfu). Clinical signs and mortality were observed for 21 days post inoculation (pi). Body weights, bacterial isolation, gross lesions and histological lesions were recorded on days 1, 3, 5,7,14 and 21pi. The inoculated chicks in A and B groups showed clinical signs of depression, anorexia, ruffled feathers, vent pasting and diarrhea starting from day 1pi. Lifting of wings from thorax was observed in group A only at day 5 and 7pi. The chicks in MA and MB groups that died during experiment showed all the clinical sings before death. There was no significant difference ($p>0.05$) in body weight gain among the inoculated and the control groups. The growth index value (0.035) for all the groups remained increased. The mortality caused by SE PT3A and PT35 was 10% and 5%, respectively. About 20-10% inoculated sacrificed and all the dead birds showed gross lesions of enlarged livers, fibrinous perihepatitis and pericarditis which was supported by histopathology. The *Salmonella* was isolated from the cultured samples of chicks inoculated with SE PT3A and SE PT35 throughout the experiment period with the individual variation of chicks and samples. It was concluded that newly hatched SPF chicks are susceptible to PT3A and PT35 infections. These phage types are mild to moderately pathogenic for SPF chicks.

Key words: Salmonella *Enteritidis*, SE PT3A, SE PT35, SPF chicks, colony forming units.

INTRODUCTION

Salmonella enterica serovar Enteritidis (S. Entiritidis) is the most common *Salmonella* serovar infecting poultry and humans worldwide (van Duijkeren *et al.,* 2002; Patrick *et al.,* 2004). Poultry is the main reservoir for this classic food borne pathogen and it has the ability to survive for long time in environments including poultry houses (Zamri-Saad and Saleha, 2006). Hence, its outbreaks are mostly associated with consumption of contaminated chicken meat, eggs or poultry products (Kimura *et al.,* 2004; Patrick *et al.,* 2004; EFSA 2009) which results in illness, hospitalizations and deaths in humans. The potential to spread through various sources has been widely reported (Gatto *et al.,* 2006; Zamri-Saad and Saleha, 2006). The SE infection is mostly symptomless, but can cause clinical illness and systemic infection in very young or stressed chicks (Desmidt *et al.,* 1997; Barrow and Wallis, 2000).

In 1980's SE outbreaks dramatically increased globally and the pathogen emerged as serious threat for poultry industry and public health (Rodrigue *et al.*, 1990; Hogue *et al,*, 1997). Since then the infections continued increasing over time, worldwide (Herikstad *et al.,* 2002; Altekruse *et al.,* 2006; CDC, 2006) and still continues to rise even though the overall incidence of *Salmonella* in general has decreased (Patrik *et al.*, 2004; CDC, 2006).

There are various phage types of SE (Ward *et al.,* 1987). The prevalent and dominant status of different phage types varies in different countries and may change in a country over time (van Duijkeren *et al.,* 2002; Fisher 2004; Pang *et al.,* 2007). There is variation in the virulence among the various phage types and even within the various isolates of the same phage type (Shivaprasad *et al.,* 1990; Barrow 1991; Alisantosa *et al.,* 2000). The variation in virulence has also been reported among the same phage types being isolated from different locations (Poppe *et al.,* 1993). Though in most of the studies PT4 is reported more virulent than other SE phage types, but this is not true in all cases (Gast and Besnon 1995; Barrow, 1991). Although, few reports on the pathogenicity of SE infection in chickens have been published (Shivaprasad *et al.,* 1990; Barrow 1991; Gast and Benson 1995; Alisantosa *et al.,* 2000; Dhillon *et al.*, 2001) however,

these are limited to few dominant phage types. To our knowledge, no study on the pathogenicity of S.Enteritidis PT3A and 35 have been reported before. Howeve, PT35 have been isolated from chickens (Icgen *et al*., 2002). This study will provide the insight for the behavior of organism in chicken and basis for the preventive and control strategies for S.Enteritidis. The objective of the study was to determine the pathogenicity of S.Enteritidis PTs 3A and 35 isolates of Malaysia after experimental infection in newly hatched SPF chicks. The pathogenicity was determined on the basis of clinical signs of disease, mortality rate, body weight gain, bacterial isolation and, observations of gross and histopathological changes.

MTTERIALS AND METHODS

***Experimental animals and Pathogenicity testing*:** Newly hatched SPF White Leghorn chicks (n=155) obtained from Malaysia Vaccine and Pharmaceutical Sdn Bhd (MVP) were brought to experimental house, Faculty of Veterinary Medicine (FVM), Universiti Putra Malaysia (UPM) Serdang, Malaysia. Five chicks were randomly separated to confirm the SPF status of chicks. These chicks were weighed and killed by cervical dislocation after taking cloacal swab and blood for bacteriology. The chicks were necropsied to observe gross lesions and samples of liver, spleen, caecal tonsils, mid-gut and caecal contents, were collected for bacteriology. Furthermore, tissue samples of liver, spleen, ilium, caeca, caecal tonsils and bursa of Fabricius were collected for hisopathology. The remaining chicks were finalized for further experimental manipulation (Ahmad *et al*., 2008).

Isolates and their phage typing: In 2005, S. Enteritidis was isolated from different samples collected from commercial chickens in different parts of Malaysia. The phage types of isolates were determined at the Laboratory of Enteric Pathogens Centre for Infectious Institute, 61 Collindale Avenue, London, United Kingdom. The PTs were designated and stocked at Pathology laboratory, FVM, UPM for further research. The SE PT3A (UPM-0541) was isolated from faecal sample of the apparently healthy commercial broiler chicken in Melaka, Malaysia, whereas the SE PT35 (UPM-0525) was isolated from liver of the apparently healthy commercial broiler chicken in Johor, Malaysia. The frozen bacterial stock were cultured on nutrient agar (Oxid, UK) and incubated at 37°C for 24 hours. The same was repeated to refresh the culture. The refreshed isolates from Nutrient Agar were identified and confirmed. The confirmed isolates were lawn cultured onto blood agar (Oxid, UK) and incubated at 37°C for 24 hours. Fresh inoculums of 10^8 cfu/ mL were prepared according to Mc Farland standard in normal saline to inoculate 0.1mL /chick (Ahmad *et al.,* 2008).

***Experimental design*:** The SPF chicks were divided into six groups. Three sacrificed groups A, B and C of 30 chicks each served to observe clinical signs, body weight gain, bacteriology and pathology .Whereas, the three mortality groups MA, MB and MC of 20 chicks each were monitored for mortality. The chicks in groups A and MA, and groups B and MB were challenged orally, with 0.1mL containing 10^7 cfu of SE PT3A (UPM-0541) and SE PT35 (UPM-0525), respectively. The un-inoculated groups C and MC served as negative controls. The chicks in groups A and MA, B and MB and, C and MC were kept separately in 3 separate rooms and each group in a separate cage. The chicks were provided with fresh water and antibiotic free feed *ad libitum* post inoculation.

Table 1: Experimental Design

Group ID	Description of groups	Time post inoculation (Days)							Total Chicks Group
		0	1	3	5	7	14	21	
		Number of chicks sacrificed							
A	Sacrificed group inoculated with SE PT3A (UPM-0541)	-	5	5	5	5	5	5	30
MA	Mortality group inoculated with SE PT3A (UPM-0541)	-	-	-	-	-	-	-	20
B	Sacrificed group inoculated with SE PT35 (UPM-0525)	-	5	5	5	5	5	5	30
MB	Mortality group inoculated with SE PT35 (UPM-0525)	-	-	-	-	-	-	-	20
C	Sacrificed Un-inoculated control group	5	5	5	5	5	5	5	35
MC	Un-inoculated mortality group	-	-	-	-	-	-	-	20

The chicks were monitored at least twice daily for clinical signs and mortality. In case of mortality in sacrificed or mortality group, chicks were necropsied and examined for gross lesions and collection of samples for bacteriology and histopathology similar as described below for sacrificed groups. On day 1, 3, 5, 7, 14 and 21 post inoculation (pi), five chicks were taken randomly from each group A, B and C. The chicks were weighed. After taking cloaceal swab and blood samples for bactriology the chicks were sacrificed humanely by cervical dislocation and necropsy was performed to observe gross lesions and collection of samples for bacteriology and histopathology. The samples from liver, spleen, caecal contents, caecal tonsils and mid-gut contents were collected from each chicken individually for bacterial isolation and identification. Similarly the

samples of liver, spleen, ilium, caeca, caecal tonsils and bursa of Fabricius were collected from each chicken individually for hisopathology (Table 1).

Clinical signs, mortality and body weight: The clinical signs of disease and mortality were observed in all the six groups at least twice daily and were recorded if any. However, the mortality in MA, MB and MC groups was considered to assess the mortality rate. The chicks in sacrificed groups A, B and C were weighed individually before sacrificed on days 1, 3, 5, 7, 14 and 21pi and the data was statistically analyzed.

Bacterial isolation and identification: The samples from cloacal swabs, blood, liver, spleen, caecal contents, caecal tonsils and mid-gut contents were collected from each chick individually for bacterial isolation and identification. The samples were individually collected in Rappapart Vassiadis (Oxid, UK) and incubated at 37°C for 18-24 hours. Then were streaked onto Brilliant Green Agar (BGA) and Xylose-Lysine-Desoxycholate Agar (XLD) and incubated at 37°C for 24 hours. Suspected black colonies from XLD and pink colonies from BGA were further inoculated for biochemical tests by Triple Sugar Iron (TSI and urease test. The TSI positive and urease test negative samples were considered positive for SE (Ahmad *et al.,* 2008).

Histopathology: The tissue samples from liver, spleen, bursa of Fabricius, ilium, caecal tonsil and caecum were aseptically collected from every chick in a bottle containing 10 % buffered formalin and kept for 24-36 hours for fixation. Tissues were further processed using standard histological techniques (Islam *et al.*, 2006). Briefly, the tissues were trimmed post fixation with scalpel blade into about 5mm pieces and were placed into embedding cassettes. The tissues were processed over night. For processing, the embedding cassettes containing tissues were placed into an automatic tissue processer (Leica, ASP300, Germany) for dehydration in a graded alcohol (30 %, 40 %, 50 %, 70 %, 90 %, and 100 %) and then cleared by two changes of 100% xylene. Then, the tissue samples were embedded in paraffin wax using semi-automatic tissue embeddor (Leica EG1160, Germany). The paraffin embedded tissues were then trimmed by microtome (Leica RM2155, Germany) at the thickness of 15 μm followed by 5 μm. After trimming the tissues were sectioned at a thickness of 4 μm. The ribbons of sectioned tissue were floated on the surface of warm water at 56^0C in a thermostatically controlled water bath (Leica H1220, Germeny). The tissue sections were taken on a glass slide from water and allowed to dry for 15-20 minutes by placing the slides in vertical position on a simple wooden rack. After drying the slides were placed on a hot plate (the tissue section not touching the plate) at 57 ^{0}C for 10-15 minutes for adhesion of the tissue and the de-waxing. The slides were labeled and stained using Haematoxylin and Eosin (HE). After staining, the slides were allowed to dry over night. Then cover slips were fixed with DPX and slides were examined under the light microscope (Islam *et al.,* 2006).

Statistical analysis: The data was analysed using Tukey's (HSD) pairwise multiple comparison procedure to determine the nature of significant effects on body weight gain (Daniel, 1991). The growth index for all the three groups was calculated as described by Waldbauer (1968).

RESULTS

Clinical signs: No abnormal clinical signs of disease were observed in both of the control groups (C and MC) throughout the experiment period. About 15-20% of the chicks inoculated with SE PT3A and SE PT35, and the 100% chicks those died during experiment showed the clinical signs from day 1 pi. The chicks inoculated with SE PT3A and SE PT35 showed the abnormal clinical signs of depression, anorexia, ruffled feather, vent pasting and diarrhea. The lifting of wings from thorax with respiratory distress was also observed in SE PT 3A inoculated chicks (Figure 1). There was variation in different clinical signs and the severity during the experiment period (Table 2).

Mortality: The mortality was recorded during first week pi. The 10% (2/20) mortality was observed in the SE PT 3A challenged mortality group (MA) chicks. One chick died on day 3 pi and the other on day 4 pi. No chick died in sacrificed group A. The 5% (1/20) mortality was observed in the SE PT 35 challenged mortality group (MB) on day 4 pi. Also one chick died in sacrificed group (B) on day one pi. No mortality was observed in un-inoculated controls throughout the experiment period.

Body weight gain: There was no significant difference ($p>0.05$) in weight gain among the chicks inoculated with SE PT3A, SE PT 35 and the un-inoculated control group (Table 3). All the three groups (A, B and C) of chicks showed same growth index (0.035) during the experiment period.

Bacterial isolation: SE was isolated from all the cultured samples from dead chicks. There was individual variation for the isolation of *Salmonella* from chicks inoculated with SE PT3A (Table 4) and SE PT35 (Table 5). The mean isolation of SE PT3A was 60% from faecal swab, 27% from heart blood, 73% from spleen, 67% from liver, 63% from mid-gut contents, 60% from caecal contents and 57% from caecal tonsils. Whereas it was 37% from faecal swab, 7% from heart blood, 37% from spleen, 30% from liver, 53% from mid-gut contents, 47% from caecal contents and 40% from caecal tonsils in chicks inoculated with SEP T35.

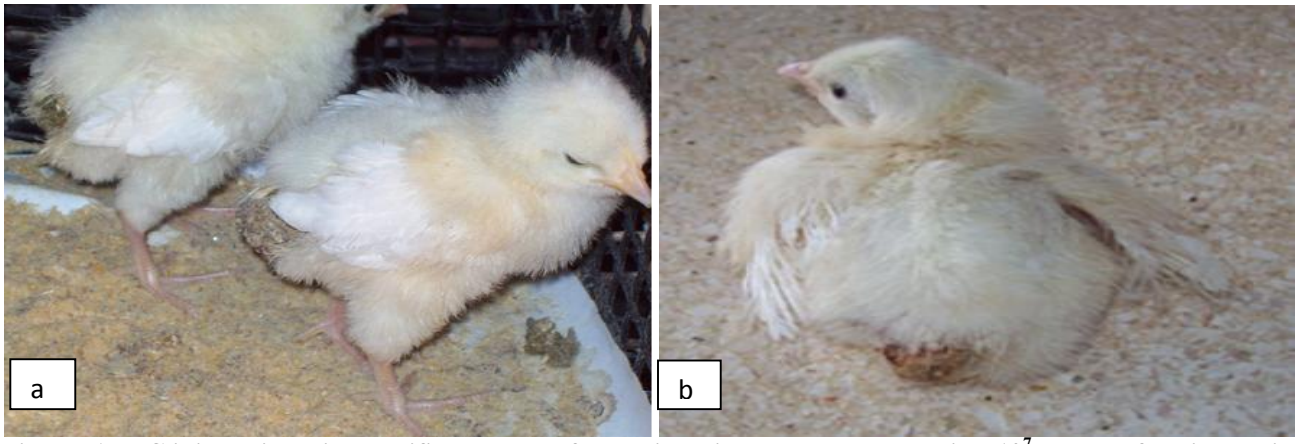

Figure 1: Clinical signs in specific-pathogen-free chicks inoculated orally with 10^7 colony forming units *Salmonella* Enteritidis phage type 3A

a) Clinical signs of depression, vent pasting and ruffled feathers at day 3pi. b) Vent pasting, lifting of wings from thorax and respiratory distress at day 5 pi.

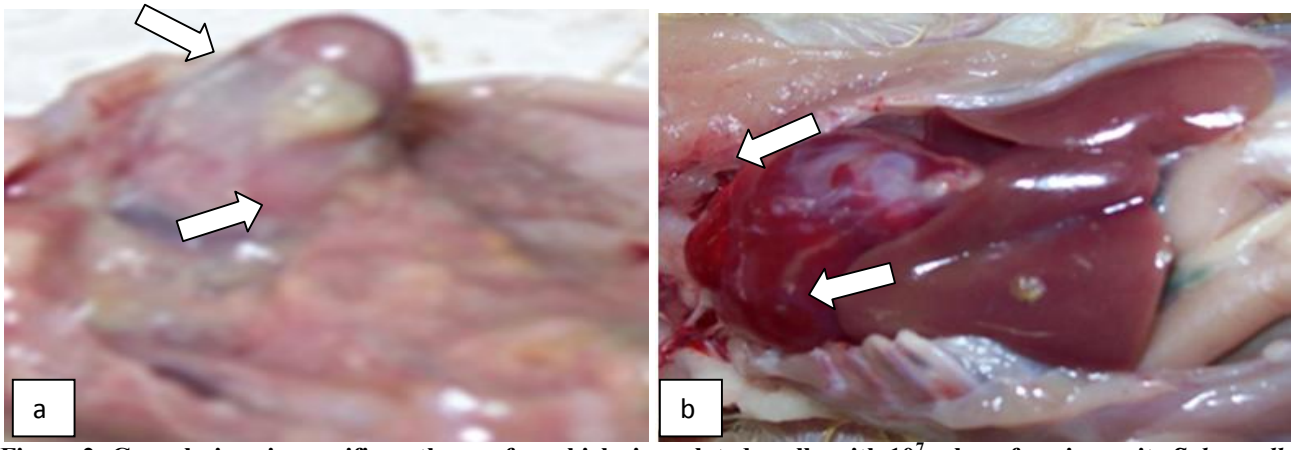

Figure 2: Gross lesions in specific-pathogen-free chicks inoculated orally with 10^7 colony forming units *Salmonella* Enteritidis phage type 3A

a) Fibrinous heart and liver at day 4 pi. b) Fibrinous heart and liver at day 21 pi.

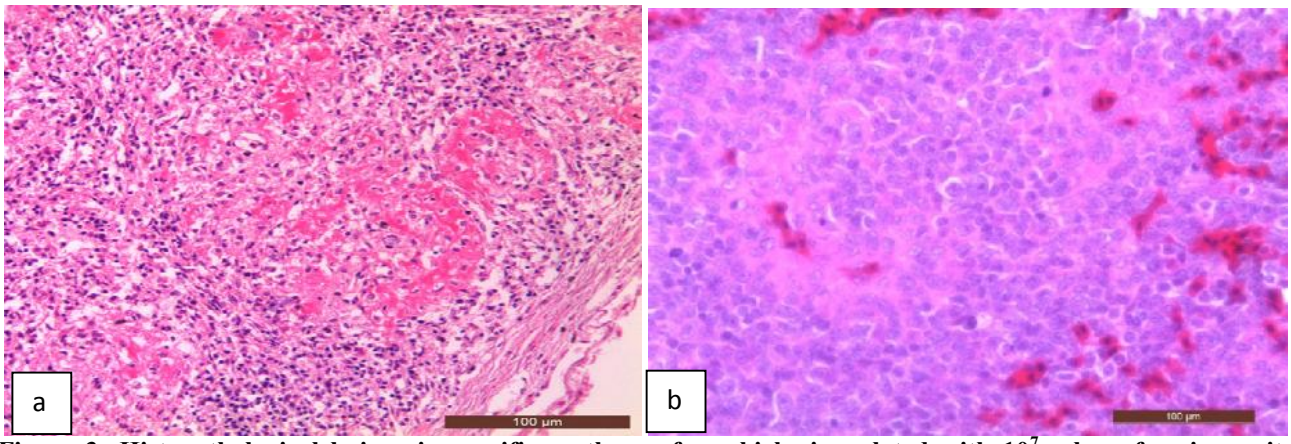

Figure 3: Histopathological lesions in specific- pathogen-free chicks inoculated with 10^7 colony forming units *Salmonella* Enteritidis phage type 3A

a) Degeneration and necrosis in spleen of chick died on day 4 pi. b) Fatty changes, degeneration and widespread necrosis in liver on day 21 pi. HE, a) Bar= 100µm b) Bar= 100µm.

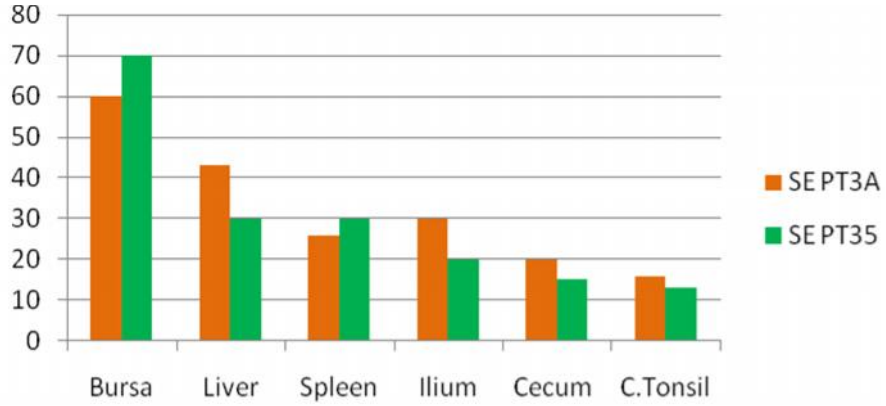

Figure 4: Percent microscopic lesions in different organs of the specific-pathogen-free -chicks inoculated with 10^7 colony forming units *Salmonella* Enteritidis phage types 3A and 35.

Table 2: Percent clinical signs of disease in newly hatched specific-pathogen-free chicks orally inoculated with 10^7 cfu of *Salmonella* Enteritidis phage types 3A and 35.

Time pi (Days)	SE PT (Group)	Percent clinical sign (No of chicks showed sign/ Total no of chicks in the group)					
		Depression	Anorexia	R.Feathers	V. pasting	Diarrhoea	Lif wings
1	3A (A)	40%(12/30)	30%(9/30)	40% (12/30)	23% (7/30)	7% (2/30)	-
	35(B)	27% (8/30)	27%(8/30)	40% (12/30)	20% (6/30)	7% (2/30)	-
3	3A (A)	36% (9/25)	24%(6/25)	48% (12/25)	16% (4/25)	12%(3/25)	-
	35(B)	21% (5/24)	21% 5/24)	33% (8/24)	21% (5/24)	8% (2/24)	-
5	3A (A)	35% (7/20)	35(7/20)	55% (11/20)	20% (4/20)	10 (2/20)	5% (1/20)
	35(B)	32% (6/19)	26%(5/19)	42% (8/19)	5% (1/19)	5% (1/19)	-
7	3A(A)	40% (6/15)	27%(4/15)	60% (9/15)	27% (4/15)	13%(2/15)	7% (1/15)
	35(B)	29% (4/14)	21%(3/14)	57% (8/14)	14% (2/14)	0% (0/14)	-
14	3A (A)	10% (1/10)	0% (0/10)	70% (7/10)	30% (3/10)	0% (0/10)	-
	35(B)	0% (0/9)	0% (0/9)	56% (5/9)	11% (1/9)	0% (0/9)	-
21	3A (A)	0% (0/5)	20% (1/5)	80% (4/5)	0% (0/5)	20% (1/5)	-
	35(B)	0% (0/4)	0% (0/4)	75% (3/4)	25% (1/4)	25% (1/4)	-

Table 3: Mean body weight (gm/chick ±SE) of newly hatched specific-pathogen-free chicks orally inoculated with *S*almonella Entritidis phage types 3A and 35, and un-inoculated control

Treatment	Time after inoculation (days)						Growth Index
	1	3	5	7	14	21	
SEPT 3A	37.93 ±0.54$^{n.s}$	39.63±1.78$^{n.s}$	43.86±2.83$^{n.s}$	51.50± 1.08$^{n.s}$	70.86±8.88$^{n.s}$	145.43±11.22$^{n.s}$	0.035
SEPT 35	37.20±0.35	38.50±1.53	48.14±1.49	52.28±2.1.01	87.96±7.23	141.28±9.50	0.035
Control	36.08±0.81	39.42±1.23	46.47±2.11	51.14±0.95	85.53±3.42	131.22±2.91	0.035
CV	-	-	-	-	-	-	

Means within columns with the same letter(s) are not significantly different (p=0.05, Tukey HSD). n.s = not significant, CV= critical value for comparison

Table 4: Isolation of *S*almonella Enteritidis phage type 3A from different organs of the orally inoculated newly hatched specific-pathogen-free chicks

Samples	Post inoculation time (days)					
	1	3	5	7	14	21
	Percent positive samples at post inoculation time (days)					
Cloacal swab	40 (2/5)	40 (2/5)	60 (3/5)	80 (4/5)	80 (4/5)	60 (3/5)
Heart blood	40 (2/5)	40 (2/5)	60 (3/5)	0 (0 /5)	20 (1/5)	0 (0/5)
Spleen	80 (4/5)	80 (4/5)	100 (5/5)	40 (2/5)	60 (3/5)	80 (4/5)
Liver	80 (4/5)	100 (5/5)	80 (4/5)	40 (2/5)	80 (4/5)	80 (4/5)
M.gut contents	80 (4/5)	60 (3/5)	100 (5/5)	60 (3/5)	40 (2/5)	40 (2/5)
Cecal contents	20 (1/5)	60 (3/5)	80 (4/5)	80 (4/5)	40 (2/5)	80 (4/5)
Caecal tonsil	60 (3/5)	40 (2/5)	40 (2/5)	80 (4/5)	60 (3/5)	60 (3/5)

Table 5: Isolation of *S*almonella Enteritidis phage type 35 from different organs of the orally inoculated newly hatched specific-pathogen-free chicks

Samples	Post inoculation time (days)					
	1	3	5	7	14	21
	Percent positive samples at post inoculation time (days)					
Cloacal swab	80 (4/5)	80 (4/5)	20 (1/5)	20 (1/5)	0 (0/5)	20 (1/5)
Heart blood	20 (1/5)	20 (1/5)	0 (0/5)	0 (0/5)	0 (0/5)	0 (0/5)
Spleen	60 (3/5)	40 (2/5)	60 (3/5)	0 (0/5)	0 (0/5)	60 (3/5)
Liver	60 (3/5)	60 (3/5)	20 (1/5)	0 (0/5)	20 (1/5)	20 (1/5)
M.gut contents	60 (3/5)	100 (5/5)	80 (4/5)	20 (1/5)	40 (2/5)	20 (1/5)
Cecal contents	80 (4/5)	100 (5/5)	40 (2/5)	20 (1/5)	0 (0/5)	40 (2/5)
Caecal tonsil	40 (2/5)	100 (5/5)	40 (2/5)	40 (2/5)	20 (1/5)	0 (0/5)

Gross lesions: Unabsorbed yolk, airsaculitis, fibrinous heart, enlarged and fibrinous liver, enlarged kidneys, spleenitis and dehydration were observed in 10% of the infected chicks and in almost all the dead chicks (Figure 2).

Histopathology: A mild inflammation was observed in intestine, caeca and caecal tonsils. The infiltration of heterophils and superficial sloughing of epithelial lining of villi were observed. The degeneration, fatty changes and multifocal necrosis in hepatocytes were the most common lesions observed in liver tissues. Spleen showed degeneration and necrosis. Bursa showed more sever lesions and in more number of samples as compared to the other tissue samples used for histopathology (Figure 3). Bursitis, degeneration, necrosis and corrugation were observed in bursa tissues Overall, the lesions were more sever in SE PT3A inoculated chicks. There was variation of lesions among the different organs of a group and also variation of lesions between the both inoculated groups (Figure 4).

DISCUSSION

The study showed that SE PT3A and PT35 isolates of Malaysia were mild to moderately pathogenic for newly hatched SPF White Leghorn chicks. The SE PT 3A and PT35 did not cause significant adverse effect on body weight gain but could cause mortality and have the potential to cause clinical illness, systemic infection and, gross and histopathological lesions.

Clinical signs: The clinical signs similar to the present study were observed previously (Shivaprasad *et al.,,* 1990; Barrow, 1991; Poppe *et al.,* 1993; Desmidt *et al.,*, 1997) but with variation in severity and expression time pi. Only SE PT4 inoculated chicks showed clinical signs on days 4 and 5 when day-old chicks were inoculated with SE PT 4, 8 and 13 (Poppe *et al.,* 1993). The clinical signs were observed only in young chicks, but not in 4-week-old chicken orally inoculated with SE PT4 (Desmidt *et al.,*, 1997). Expression, severity or even the absence of clinical signs depends upon many factors including the phage types and the dose rates (Barrow 1991; Poppe *et al.,* 1993). The decline in severity of clinical signs from day 14 pi may be assigned to age of chicks. Presence of about 80% infected chicks as silent carrier could be a serious threat for poultry industry and public health.

Mortality: The infection with SE PT3A and PT 35 caused 10% and 5% mortality, respectively during first week of age only. Previously, 8% mortality was recorded in 1-day-old chicks inoculated with SE PT5 (Dhillon *et al.,* 2001). Similarly Desmidt *et al* (1997) reported 8% mortality with SE PT4 and Alisantosa (2000) reported 3% mortality with SE PT8 (90-631) in one-day-old chicks inoculated orally. In contrast to the present study, high mortality rates of 96% with SE PT4, 55% with SE PT6 and 60% with SE PT8 have been reported in one-day-old chicks (Barrow, 1991). The difference in mortality rates in the present study and previous studies may be due to the different phage types used for the studies as different phage types of SE and even same phage types vary in their virulence (Shivaprasad *et al.,* 1990; Barow 1991; Poppe *et al.,* 1993; Gast and Benson 1995; Alisantosa *et al.,* 2000; Dhillon *et al.,* 2001).

Body weight gain: All the chicks gained weight during the experimental period and there was no significant difference ($p>0.5$) among SE PT3A, SE PT 35 and the controls. The results of the study are in contrast to previous studies (Dhillon *et al.,* 1999; Alisantosa *et al.,* 2000), that could be assigned to different phage types studied in the present and previous studies.

Bacterial isolation: The pathogen was detected in all the samples taken from dead birds, but was not recovered in every challenged chick and every sample. The individual variation was also observed in isolation of bacteria from various samples. The bacterial isolation was relatively higher in all sampled organs in SE PT3A than that of SE PT35 inoculated chicks. Previously Poppe *et al.,* (1993), Dhillon *et al.,* (1999) and Alisantosa *et al.,* (2000) isolated *Salmonella* from various samples. They further observed variation in bacterial isolation of different phage type tested. The present organ colonization by SE not in every challenged bird is in conformity with the

previous studies (Duche-Suchaux *et al.,* 1995; Asheg *et al.,* 2001). Similarly detection of *Salmonella* in different organs depends on invasive potential of a phage type (Barrow 1991) which explains the pathogenicity of a phage type. Detection of SE in liver and spleen in dead chicks suggested that death originated from Salmonellosis (Duche-Suchaux *et al.,* 1995).

Gross lesions: The results of gross lesions indicated that these PTs are less invasive and hence mildly virulent. Similar lesions have been observed with variation in number of chicks and organs showing lesions in previous studies conducted with S.Entritidis PTs 4, 8 and 23 (Alisantosa *et al.,* 2000). The gross lesions present in all infected chicks that died during study suggest that septicemia is associated with fatal avian S.Enteritidis infections (Gorham *et al.,* 1994).

Histopathology: The gross and microscopic lesions correlate with the bacterial isolation and clinical signs of disease. The sloughing of superficial layers of villi reveals the damage to the integrity of intestinal epithelium that results into translocation of bacteria to other tissues. If *Salmonella* are not cleared by host immune system the intestinal colonization occurs that can further lead to invasion and colonization of other organs including liver and spleen and results in lesions in invaded tissues (Alisantosa *et* al., 2000; Ahmad *et al*., 2008). The lesions in the liver, spleen, caecum and caecal tonsils explain the invasive potential of SE PTs and their respective pathogenicity. The presence of heterophils in luminal surface of intestine witnessed the inflammatory reaction caused by SE infections (Henderson et al. 1999) The similar microscopic lesions have been reported previously but for SE PTs other than we used in the present study (Gorham *et al*., 1994; Dhillon *et al*., 1999; Alisantosa *et al*., 2000).

Conclusion: In conclusion the study has shown that newly hatched chicks are susceptible to SEPT3A and SEPT35 infections which may cause clinical disease but the majority may act as carriers for other chicks and humans. These phage types are mild to moderately pathogenic however; SE PT3A is more pathogenic when compared to SE PT35.

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