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Mode of vertical transmission of *Salmonella enterica* sub. *enterica* serovar Pullorum in chickens

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The mode of vertical transmission was studied with local isolate of *Salmonella pullorum* in hens and cocks. Twenty (20) hens and five cocks were experimentally infected by the oral route with 2 × 10^7 (CFU) dose of *S. pullorum* organisms at 21 weeks of age in hens and 29 weeks in cocks and in control (20 hens and five cocks) no bacteria was given. The used methods were reisolation of *S. pullorum* from different organs, blood, eggs and newly hatched chicks, and detection of *S. pullorum* by PCR from testicular tissue at different time intervals of experimental period. Five birds (four hens and one cock) randomly selected and sacrificed on 6 h before inoculation (BI) and one, two, three and four weeks of post-infection (PI) from infected and control group. *S. pullorum* was reisolated from 50% eggs of experimentally infected hens. Twenty percent hatchability was lost due to experimental *S. pullorum* infection. Piped chicks were 20% and embryo mortality was 15%. *S. pullorum* was isolated from 66.66% chicks. Seventy five percent testes were positive for *S. pullorum* by culture and biochemical test. *S. pullorum* was detected by PCR at one to three weeks PI from testicular tissues. It was clear that after oral route of infection with infective dose of *S. pullorum*, the bacteria invaded digestive epithelia and ultimately entered into blood inducing bacteremia and ultimately infected different organs and produced pathological lesions. It was also confirmed that the bacteria invaded ovary and egg follicles, and this infection persisted in ovary and egg follicles and transmitted into laid eggs then to hatched chicks.

Key words: Vertical transmission, *Salmonella pullorum*, chickens.

INTRODUCTION

Pullorum disease (PD) is an acute, infectious, and fatal disease of chicks causing mortality as well as results in persistent infection and vertical transmission in layer birds (Wray and Davies, 2001; Ramasamy et al., 2012). PD in growing and mature fowl is characterized by a sudden drop in feed consumption, with ruffled feathers, and pale and shrunk combs. Other signs in laying hens are characterized by a drop in egg production, decreased fertility, and diminished hatchability. The prominent signs of PD are anorexia, diarrhoea, depression, and dehydration (Chauhan and Roy, 1996; Haider et al., 2003; Hossain et al., 2006).

Pullorum disease may be transmited horizontally and vertically. Vertical transmission may result from contamination of the ovum following ovulation, but localization of *Salmonella pullorum* in the ovules before ovulation

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also likely and probably constitutes the chief mode of transmission (Wigely et al., 2005). Embryonic dead and chick mortality in PD due to vertical transmission are the major problems, and it causes great economic losses in the poultry industry of Bangladesh. However, in Bangladesh no systematic investigation has been performed on the experimental vertical transmission of PD in laying hens. Therefore, this investigation is designed to correlate among oral routes of infection with *S. pullorum*, ovarian follicle infection, reisolation of *S. pullorum* from experimentally inoculated ovarian follicles of infected hens and eggs laid by them, and pathological lesions of ovarian follicles of experimentally inoculated infected hens. Findings obtained from this investigation would be useful for control and eradication measures of PD spread by vertical route of transmission in Bangladesh.

PD causes great economic losses every year in poultry farms and it has also zoonotic importance. The diseases can spread via meat and eggs. For exporting the poultry meat and eggs, salmonellae should be free in Bangladesh.

A few investigations on natural cases of *Salmonella* infections have been completed in Bangladesh using the methods of necropsy, histopathology and isolation of bacteria by culture; staining and sugar fermentation tests (Ahmed et al., 2008; Haider et al., 2008; Islam et al*.,* 2006). However no investigations have been performed by locally isolated *S. pullorum* organisms in experimental pathogenesis, pathology and vertical transmission in chickens. For this reason, the present study was taken first time for better diagnosis, prevention and control of this economically important pullorum disease in Bangladesh.

MATERIALS AND METHODS

The chickens of different age group were purchased from Nourish Hatchery Ltd, Shreepur, Gazipur and reared in different poultry sheds of the Department of Pathology and poultry units of the Department of Microbiology and Hygiene.

Experimental hens and cocks

A total of 40 pullets (*Salmonella pullorum* seronegative) of Isa Brown breed of 18 weeks were purchased from Nourish Hatchery Ltd., Shreepur, Gazipur, Bangladesh and 10 cocks (*Salmonella pullorum* seronegative) of RIR (Road Island Red) breed of 26 weeks old were taken from BAU Poultry farm. The birds were vaccinated against Marek's Disease, Infectious Bursal Disease, Infectious Bronchitis, Fowl Pox and Newcastle Disease obtained from Intervet, Holland. The birds were divided into two groups in which one group remained as control. At the beginning of laying the pullets were called as hens.

Bacterial infection

25 (20 hens at the age of 21 weeks and five cocks at the age of 29 weeks) birds were experimentally infected by the oral route with 2×10^7(CFU, Colony Forming Unit) dose of *Salmonella enterica* sub. *enterica* serovar Pullorum (Isolate No. 5) organisms in 0.5 ml broth culture with 0.5 ml of sterile phosphate buffer saline (PBS), pH 7.2, using sterile syringe (Roy et al., 2001; Wigley et al*.,* 2005). Control birds were given only 0.5 ml broth without bacteria with 0.5 ml of PBS.

Samples collection

Five (five hens and one cock) birds in each case were randomly selected and sacrificed 6 h before inoculation and one, two, three and four weeks PI. A total of 25 (20 hens and 5 cocks) birds were used for the control group, and necropsies were carried out in similar a similar matter as the infection groups. Different types of samples were collected as described earlier. Eggs, ova and parts of female reproductive organs were collected for the isolation of microorga-nisms (Wigley et al*.,* 2005).

Re-isolation of *Salmonella pullorum*

Collected samples (crop, liver, lung, heart, duodenum, cecum, kidney, bile and spleen) were weighed and placed in a tube containing 1 ml of sterile phosphate - buffered saline (PBS) solution. The colony-forming units of *S. pullorum* were counted using standard microbiological methods (Haider et al*.,* 2008; Haider et al., 2012).

For each bird in each group of 6 h before inoculation and 1, 2, 3 and four weeks post-infection, 1 ml of blood was collected and *S. pullorum* was re-isolated using previously described methods (Haider et al*.,* 2008; Haider et al., 2012). *S. pullorum* was reisolated from ovary, ovarian follicle, oviduct, uterus, vagina and testis as described previously (Wigely et al*.,* 2005). Laid eggs were collected and soaked in Lugol's iodine for 20 min. The presence of *S. pullorum* was confirmed by standard procedures (Cheesbrough, 2000; Haider et al., 2003; Haider et al., 2012).

Hatching of eggs

Standard procedure was followed for preparation of incubator and setting eggs in the incubator. The candling of eggs was also done at 10 and 18 days of incubation with the help of electric Candler for dead embryos (Carol and Gregg, 2002):

The unhatched eggs were broken up to confirm the death of embryos.
The chicks that piped but did not come out of the shell were considered piped chicks.
The chicks that came out of the shell on 21 days of incubation that were recorded as hatched chicks.

Fertility of eggs was calculated by using the following formula:

$$\text{Fertility (\%)} = \frac{\text{Total number of fertile eggs}}{\text{Total number of eggs set}} \times 100$$

Hatchability of eggs was calculated on the basis of egg set and on fertile eggs, and these were calculated by the following formulae (Carol and Gregg, 2002):

$$\text{Hatchability on eggs set (\%)} = \frac{\text{Total number of chicks hatched}}{\text{Total number of eggs set}} \times 100$$

Table 1. Average number of CFU/g of isolated and identified of *Salmonella pullorum* from different organs of experimentally infected hens.

Organ	BI 6 h	PI 1	PI 2	PI 3	PI 4
1. Crop	00(0/4)	58.72×10^4(4/4)	25.81×10^3(4/4)	25.68×10^2(3/4)	14.96×10^1(2/4)
2. Liver	00(0/4)	64.58×10^5(4/4)	65.79×10^4(4/4)	55.98×10^3(4/4)	44.28×10^3(3/4)
3. Heart	00(0/4)	21.34×10^3(4/4)	40.16×10^3(4/4)	48.66×10^2(3/4)	14.71×10^2(3/4)
4. Lungs	00(0/4)	44.85×10^4(4/4)	$39.02.2 \times 10^4$(4/4)	66.95×10^3 (4/4)	24.26×10^2(4/4)
5. Duodenum	00(0/4)	20.98×10^5(4/4)	47.51×10^4 (4/4)	36.54×10^4(4/4)	43.56×10^3(4/4)
6. Cecum	00(0/4)	81.11×10^5(4/4)	73.93×10^5(4/4)	64.47×10^4 (4/4)	80.77×10^3(4/4)
7. Bile	00(0/4)	47.88×10^3(2/4)	14.29×10^3(1/4)	00(0/4)	00(0/4)
8. Kidney	00(0/4)	39.72×10^3(4/4)	13.58×10^3(4/4)	18.84×10^2(2/4)	19.26×10^1 (2/4)
9. Spleen	00(0/4)	89.25×10^5(4/4)	93.34×10^5(4/4)	62.21×10^3(4/4)	51.25×10^3(4/4)

$$\text{Hatchability on fertile eggs (\%)} = \frac{\text{Total number of chicks hatched}}{\text{Total number of fertile eggs}} \times 100$$

Embryonic mortality was calculated by the following formula

$$\text{Embryonic mortality (\%)} = \frac{\text{Total number of dead embryos}}{\text{Total number of fertile eggs}} \times 100$$

S. pullorum was re-isolated from liver, lungs, heart, ceca and yolk materials from newly hatched chicks and dead embryos as described by Wigely et al*.,* 2005.

Detection of *Salmonella pullorum* by PCR

Testes were collected in sterile poly bag with PBS at different time intervals. Testicular tissue was used for the detection of microorganisms by PCR (Raln et al*.,* 1992).

RESULTS

Re-isolated *S. pullorum* produced pink colour colonies on BGA, and CFU/g of tissues was counted and recorded. *S. pullorum* showed red - pink - white opaque coloured colonies surrounded by brilliant red zones in BGA. In Gram's staining, the morphology of the isolated bacteria was small, rod shape, Gram negative and single or paired in arrangement. *S. pullorum* produced an alkaline (red) slant and acid (yellow) butt, with gas bubbles in the agar and a blackening due to H_2S production observed the acid reaction of the butt in TSI agar. *S. pullorum* showed lysine decarboxylation, with a deeper purple (alkaline) slant and alkaline or neutral butt with slight blackening due to H_2S production in LI agar. The isolated organisms fermented dextrose, manitol and xylose with gas production and did not ferment lactose, sucrose, dulcitol, inositol and maltose. The organisms were positive to MR test and were negative to indole and VP test. Limited movement was observed in the isolated organisms.

Re-isolation of *Salmonella pullorum* from different organs of hens

Reisolation of *S. pullorum* from different organs was variable in different time schedule (Table 1). Of the total tissue collected, 93.75% liver, 100% lungs, 100% duodenum, 100% ceca and 100% spleen were positive for *S. pullorum* at 1 to 4 weeks PI. The *S. pullorum* was re-isolated from 81.25% crop, 87.5% heart, 18.75% bile and 75% kidney throughout the study period. The highest number of *S. pullorum* re-isolated was 64.58×10^5 in liver at 1 week PI and the lowest was 14.96×10^1 in crop at 4 weeks PI. The average numbers of CFU/g of re-isolated S. pullorum from different time intervals are shown in Table 1. Control group was free from *S. pullorum* in culture during the study period.

Reisolation of *Salmonella pullorum* from blood

The average number of CFU/ml of *S. pullorum* re-isolated from blood shown in Table 2. The blood sample of four hens out of four (4/4) at 1 and 2 weeks PI, three hens out of four (3/4) and one hens out of four (1/4) at 3 weeks and four weeks PI, respectively, were positive for *S. pullorum*. The highest number of CFU/ml was 13.55×10^3 at 1 PI and the lowest was 13×10^2 at 4 weeks PI. No *S. pullorum* was found in the control group. Colony characters and results of biochemical tests of re-isolated *S. pullorum* were found similar which were described earlier.

Re-isolation of *S. pullorum* from female reproductive organs

Salmonella pullorum was re-isolated from ovary (100%), ovarian follicle (100%), oviduct (68.75%), uterus (56.25%) and vagina (75%) of female reproductive organs after experimental infection (Table 3). No *S. pullorum* was found in control group hens. Re-isolated *S.*

Table 2. Re-isolation and average number of CFU/ml of isolated and identified of *Salmonella pullorum* from blood of experimentally infected hens.

Time schedule of PI	Infection group		Control group	
	Number	Mean CFU	Number	Mean CFU
BI 6 h	0/4	00	0/4	00
PI 1	4/4	13.55×10^3	0/4	00
PI 2	4/4	8.43×10^3	0/4	00
PI 3	3/4	33.76×10^2	0/4	00
PI 4	1/4	13×10^2	0/4	00

Percentage calculated from 1 PI to 4 weeks PI

Table 3. Average number of CFU/g of isolated and identified *Salmonella pullorum* from female reproductive organs in experimentally infected hens.

Organ	BI 6 h	PI 1	PI 2w	PI 3	PI 4	Total (%)
Ovary	00(0/4)	41.05×10^5(4/4)	64.6×10^4(4/4)	33.05×10^3(4/4)	40.93×10^4(4/4)	100
Ovarian follicle	00(0/4)	67.75×10^4(4/4)	47.61×10^4(4/4)	31.67×10^3(4/4)	49.5×10^3(4/4)	100
Oviduct	00(0/4)	39.65×10^4(4/4)	25.17×10^3(3/4)	40.67×10^3(2/4)	27.54×10^3(2/4)	68.75
Uterus	00(0/4)	40.11×10^5(3/4)	14.36×10^3(3/4)	19.16×10^3 (2/4)	30.81×10^3(1/4)	56.25
Vagina	00(0/4)	51.15×10^3(4/4)	07.2×10^3(4/4)	43.12×10^3 (3/4)	13.13×10^3(1/4)	75

Percentage calculated from 1 PI to 4 weeks PI.

Table 4. Average number of CFU/g of isolated and identified *Salmonella pullorum* from different organs of cocks after experimental infection.

Organ	BI 6 h	PI 1	PI 2w	PI 3	PI 4	Total (%)
Liver	00(0/1)	69.83×10^3(1/1)	158.62×10^3(1/1)	37.05×10^3(1/1)	40.93×10^2(1/1)	100
Lung	00(0/1)	60.89×10^3(1/1)	86.90×10^3(1/1)	31.67×10^3(1/1)	49.5×10^2(1/1)	100
Heart	00(0/1)	12.24×10^3(1/1)	23.31×10^3(1/1)	41.67×10^2(1/1)	00(0/1)	75
Spleen	00(0/1)	105.41×10^3(1/1)	163.36×10^3(1/1)	89.16×10^3 (1/1)	30.81×10^2(1/1)	100
Cecum	00(0/1)	236.88×10^3(1/1)	118.68×10^3(1/1)	103.12×10^3 (1/1)	83.13×10^3(1/1)	100
Testis	00(0/1)	35×10^2(1/1)	16.4×10^3(1/1)	19.32×10^2(1/1)	00(0/1)	75

pullorum produced pink colour colonies on BGA, and CFU/g of tissues was counted and recorded.

Re-isolation of *S. pullorum* from different organs of cocks

Re-isolation rate of *S. pullorum* from different organs was variable in different time schedules (Table 4). 100% liver, 100% lungs, 75% heart, 100% cecum, 100% spleen and 75% testes were positive for *S. pullorum* at 1 to 4 weeks PI. Control group was free from *S. pullorum* in culture during the study period. Re-isolated *S. pullorum* produced pink colour colonies on BGA, and CFU/g of tissues was counted and recorded.

Re-isolation of *Salmonella pullorum* from laid eggs

Average number of CFU/g of isolated and identified of *S. pullorum* from eggs of experimentally infected hens are showed in Table 5. Isolation rate of *S. pullorum* from outer shell of laid eggs was 95%, the highest positive while, the second highest was 50% in the egg yolk. The lowest isolation rate of *S. pullorum* was in inner shell and egg albumin, which were 45 and 35%, respectively. Throughout the study period *S. pullorum* was re-isolated from 50% of the laid eggs and no *S. pullorum* was found in control hen's laid eggs. Re isolated *S. pullorum* produced pink colour colonies on BGA, and CFU/g of tissues was counted and recorded.

Hatching of eggs

Hatching information of eggs after experimental infection with *S. pullorum* is shown in the Table 6. Fertility was 65% in the infection group and 85% in control group

Table 5. Average number of CFU/g of isolated and identified *Salmonella pullorum* from eggs of experimentally infected hens.

Source	BI 6 h	PI 1	PI 2w	PI 3	PI 4	Total (%)
Outer shell	00(0/5)	11.1×10^6(4/5)	05.52×10^6(5/5)	04.38×10^3(5/5)	17.82×10^3(5/5)	95
Inner shell	00(0/5)	12.59×0^3(2/5)	01.78×10^3(3/5)	01.36×10^3(2/5)	04.26×10^3(2/5)	45
Egg albumin	00(0/5)	41.42×0^3(2/5)	01.99×10^3(2/5)	10.69×10^3(2/5)	13.82×10^2(1/5)	35
Egg yolk	00(0/5)	44.1×10^3(3/5)	10.3×10^3(3/5)	78.5×10^5(2/5)	24.36×10^3(2/5)	50

Table 6. Hatching indices of chicks after experimental infection with *Salmonella pullorum* (n=20).

Hatching index	Infection group (%)	Control group (%)
1. Fertility	65	85
2. Hatchability		
a. Hatchability on eggs set	50	85
b. Hatchability on fertile eggs	77	100
3. Embryonic mortality	15	00
4. Dead-in- shell	15	00
5. Piped chicks	20	05

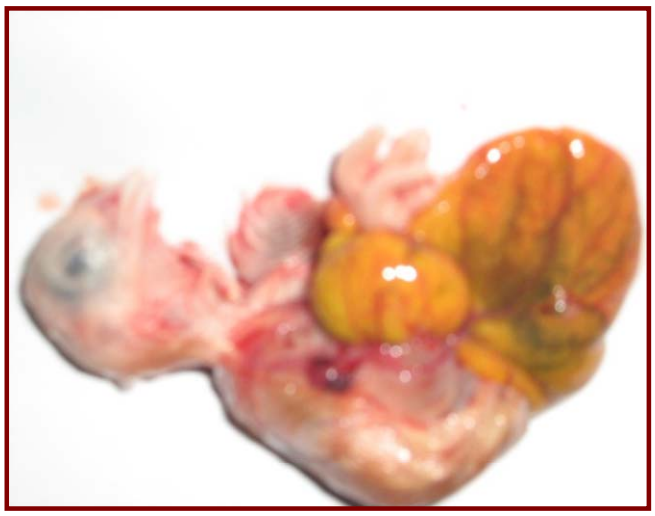

Figure 1. Showing the embryonic death of chicks with unabsorbed and coagulated egg-yolk after experimental infection with *S. Pullourm*.

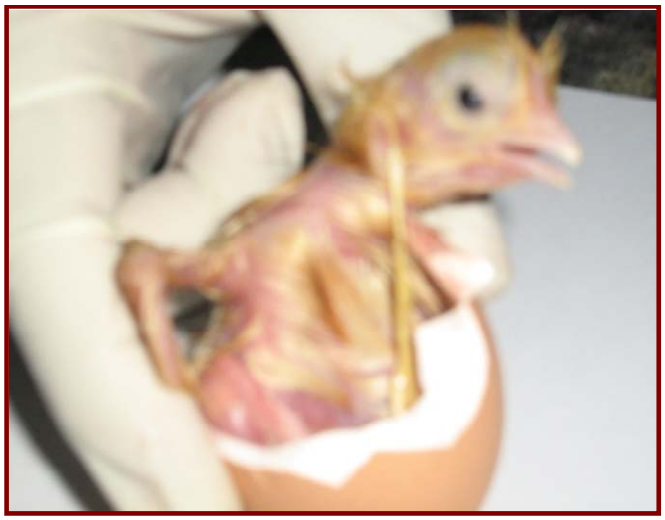

Figure 2. Showing the piped chicks after experimental infection with *S. Pullourm*.

hen's laid eggs. Hatchability of eggs set and hatchability of fertile eggs were 50 and 77%, respectively in infected eggs.

Hatchability of 100% on fertile eggs was found in the control group. The embryonic mortality (Figure 1), dead-in-shell and piped chicks (Figure 2) were 15, 15 and 20%, respectively. No embryonic mortality, no dead-in-shell and 5% piped chicks were found in the control group eggs.

Re-isolation of *S. pullorum* from different organs of newly hatched chicks and dead embryos

S. pullorum was re-isolated in 66.66% of liver, lungs, ceca and yolk materials, and 55.55% of heart from newly hatched chicks and dead embryos, respectively (Table 7). The highest CFU of *S. pullorum* was 59.23×10^6 re-isolated from yolk materials. No *S. pullorum* was found in control chicks. Re-isolated *S. pullorum* produced pink

Table 7. Average number of CFU/g of re-isolated and identified of *S. pullorum* from different organs of newly hatched chicks of infected group (n=9) and control group (n=10).

Organ	Control group	% Isolation	Infection group	% Isolation
Liver	00 (0/10)	00	49.11×10^4 (6/9)	66.66
Lungs	00 (0/10)	00	33.28×10^4 (6/9)	66.66
Heart	00 (0/10)	00	17.9×10^4 (5/9)	55.55
Ceca	00 (0/10)	00	54.4×10^6 (6/9)	66.66
Yolk materials	00 (0/10)	00	59.23×10^6 (6/9)	66.66

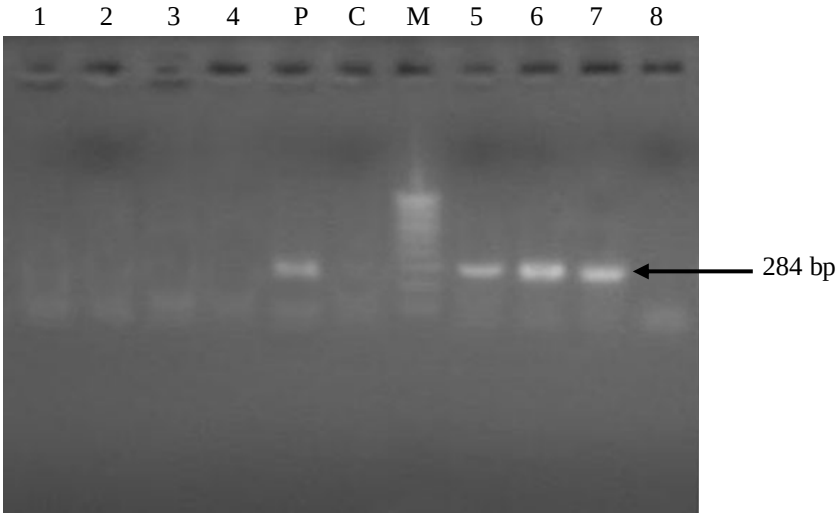

Figure 3. Electrophoresis of amplified samples of testes in control and infected cocks specific for *Salmonella pullorum* on 1.5% agarose gel. No bands are seen in lanes 1 to 4 from testes in the control group of cocks; lane P showing the 284-bp band as a positive control; lane C showing no band as a negative control; lanes 5 to 7 showing 284-bp bands from testes of infected cocks at 1 to 3 weeks PI, and lane 8 showing no band at 4 weeks PI from testes of infected cocks. Lane M showing DNA molecular Mass marker (100).

colour colonies on BGA, and CFU/g of tissues was counted and recorded.

Detection of *Salmonella pullorum* by PCR

S. pullorum was detected by PCR at 1 PI to 3 weeks PI from testicular tissue of infected cocks (Figure 3). No amplification was seen at 4 weeks PI and in the control group of cocks.

DISCUSSION

Salmonella enterica serovar Pullorum causes persistent infections in laying hens. Splenic macrophages are the main site of persistence. At sexual maturity, numbers of bacteria increase and spread to the reproductive tract which this may result in vertical transmission to eggs or chicks (Wigley et al., 2005). Eggs transmission may result from contamination of the ovum following ovulation, but localization of *S. pullorum* in the ovules before ovulation is also likely and probably constitutes the main mode of transmission (Shivaprasad, 1997). Transmission through eggshell penetration by *S. pullorum* is also reported (Shivaprasad, 1997; Williams et al*.,* 1968). In this investigation, localization of *S. pullorum* in specific cell types was not detected by immnunohistochemistry. The other findings of the present study aggress with previous reports (Shivaprasad 1997; Wigley et al., 2005; Williams et al*.,* 1968).

In this study, the birds experimentally infected at 18 weeks through orally with 2×10^7 *S. pullorum* and the re-isolation rate was 50% in laid eggs during the study period. Wigley et al. (2001), found in commercial brown-eggs layers infected orally 1×10^9 organisms of *S. pullorum* at 1 of age laid eggs 60% positive for S. *pullorum* from 18 weeks PI to 42 weeks PI but the average

positive was 6.5%. In the present study, re-isolation of *S. pullorum* was highest at 2 PI and decreased gradually at 3 weeks PI to onward while Okamura et al. (2001) recovered *S. enteritidis* increased from 50% at 4 days PI to 100% 7 days PI and viable count also increased. In the present study, 50% laid eggs was infected with *S. pullorum* but Chauhan and Roy (1996) reported 34% infected laid eggs; Shivaprasad (1997) reported 33% infected laid eggs; and Wary and Davies (2001) only a small percentage of the eggs is also likely to be infected with *S. pullorum*.

In the present study, *S. pullorum* were re-isolated from different reproductive organs of male and female, eggs and newly hatched chicks at experimental PI at different time intervals. *S. pullorum* was re-isolated from ovary (100%), ovarian follicle (100%), oviduct (68.75%), uterus (56.25%) and vagina (75%) of female reproductive organs after experimental infection at 21 weeks of age with 2×10^7 CFU of *S. pullorum* in this study. While Kinde et al. (2000) recorded ovary (58%) and oviduct (42%) positive with the field isolate of *Salmonella enteritidis* phage type 4 and Okamura et al. (2001) recovered *S. enteritidis, S. Typhimuriam* and *Salmonella Hadar* from the ovary 100, 40 and 20%, and from follicles 87.5, 10, and 13.36%, respectively at 4 days PI. *Salmonella* serovar Pullorum was recovered from 75% reproductive tracts of chickens, 80% ovary and 60% oviduct at 18 weeks PI, and 37.5% ovary and 12.5% oviduct at 22 weeks PI by Wigley et al. (2005). Ovaries (100%) and oviducts (80%) were found positive for S. *pullorum* at 20 weeks PI (Wigley et al., 2001). Michailidis et al. (2012) and Anastasiadou et al. (2013) quantified antimicrobial peptides avian β-defesins (AvβDs) in chicken ovary and vagina, respectively, during sexual maturation and Salmonella infection using real-time PCR.

Trampel (2001) reported 55% reduction of hatchability in clinical outbreaks of pullorum disease (PD) in laying hens. In the present study 23% reduction of hatchability on fertile eggs was found in infection group in comparison to control group. It is indicating that PD has the effects on hatchability but the exact mechanisms cannot be explained from this experiment. In this study, 20% fertility was reduced in comparison to control group while other authors (Shivaprasad, 1997; Trampel, 2001; Wray and Davies, 2001) reported the reduction in some percentage of fertility in PD.

Chauhan and Roy (1996), Shivaprasad (1997), Trampel (2001), Withanage et al. (2004) and Wray and Davies (2001) speculated that the infection to chicks comes from the infected eggs laid by a carrier hen. In the incubator, the hatched diseased chicks spread infection to other healthy chicks. In the present study, *S. pullorum* were re-isolated from hatched chicks of infection group of liver (66.66%), lung (66.66%), heart (55.55%), cecum (66.66%) and yolk materials (66.66%). It may be speculated that hatched chicks received infection from infected eggs laid by infected hens.

In the present study, *S. pullorum* was re-isolated from 75% testes of cocks but Wigley et al. (2005) could not re-isolate *S. pullorum* in the testes of male birds. In this experiments, it is cleared that after oral route of infection with infective dose of *S. pullorum*, the bacteria invades digestive epithelia and ultimately enters into blood causing bacteremia which is corresponded with the finding of Haider et al. (2012). From blood, bacteria are seeded into cells and tissues of different organs such as liver, lung, spleen, kidney, different parts of reproductive tracts of hens and male testes. It is also confirmed that the bacteria invades ovary and egg follicles, and this infection persists in ovary and egg follicles and transmits into laid eggs then to hatched chicks. In this study vertical transmission is known in chickens. In future for the control of *Salmonella* infections in poultry, vaccine production and sequencing of vaccine candidate in association with phylogenetic analysis of circulating *Salmonella* organisms would be performed in Bangladesh.

Conflict of interests

The author(s) have not declared any conflict of interests.

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