ISOLATION OF *SALMONELLA TYPHIMURIUM* FROM CATTLE WITH STUDY OF PATHOLOGICAL LESIONS OF SOME INTERNAL ORGANS

Ghassan A. Daeih and Zainab R.Zghair

Zoonosis unit, College of Veterinary Medicine, University of Baghdad, Iraq.

Abstract

This study was designed to isolation of *Salmonella typhimurium* from cattle and histopathological study of internal organs of infected cases. The first portion of this study included, isolation of *S. typhimurium* from 105 samples from cattle showed that isolates culture from cattle's (n=105) found to be nine of *S. typhimurium* isolates with a percentage (8.57%). Fecal samples from cattle's, cultured on pre-enrichment media peptone water, and cultured on enrichment media tetrathionate broth. then on selective media like Xylose Lysine Deoxycholate agar (XLDagar) and *Salmonella* high selective media (chromogenic media) namely BrillianceÔ Salmonella agar base. From 10 samples that suspected with *S. typhimurium* identify by conventional biochemical and Api20 E, only 9 samples was confirmed with *S. typhimurium*. Histopathological study of intestine of cow showed necrotic with distorted villi infiltration of inflammatory cells with mucosa and submucosa which is edematous and focal ulceration and thickening of blood vessel wall, sloughing of mucosa layer and thickening of sub mucosa that infiltrated with neutrophile and plasma cells in intestine and in liver appeared dilated of sinusois and hepatocytes like cords with granulation of cytoplasm of hepatocytes.

Key words : *Salmonella typhimurium*, cattle, pathological lesions.

Introduction

Salmonella is a main health problem worldwide that causes non-typhoidal salmonellosis (NTS) and typhoidal salmonellosis. These two categories of salmonellosis infect millions of cases yearly with important economic losses and even life threatening (Manoj *et al.,* 2015; Barrow and Methner, 2013). This infection originated mainly in animals such as cattle as well as asymptomatic carriers in different animal species (Martelli and Davies., 2012 ; De Knegt *et al.,* 2015). This pathogen is mainly transmitted through animal trade and along with uncooked animal food products. Furthermore, the most important sources of infection can originated from the removal processing of elementary tract during slaughtering of animal contaminated with *Salmonella*. Also animals that do not appear symptoms can be considered potential source of infection **(**Morpeth *et al.,* 2010; Aftab *et al.,* 2012; Kagambega *et al.,* 2013; Vohra *et al.,* 2019). *Salmonella* pathogen has been isolated from fecal samples of carrier animals such as cattle and calves, along with animal infected with diarrhea. The diagnosis of *Salmonella* spp. from stool samples is important and also

essential to detect carriers or reservoirs of this pathogen especially between food handlers, who are under risk diffuse the organism (Al-Helfee, 2014). Procedures for isolation of *Salmonella* include culturing of fecal sample, especially from diarrheal specimen. Programs control and checking of *Salmonella* using a specific and sensitive laboratory technique for isolation, detection and serotyping of it. Although Perfect diagnostic methods for Salmonellaare easily, reproducible, inexpensive, specific and sensitive, no single technique meets all these standards (WHO 2010).

Aim of the study isolation and detection of *S. Typhimurium* from carriers and infected cattle, and histopathological examination for internal organs of infected cases.

Materials and Methods

Bacterial isolation

Collection of samples

This study was conducted in central and south region of salahaddin governorate in

Iraq. 105 fecal sample from cattle during the period from December/ 2018 to May/2019 for all samples.

One hundred fecal samples were collected from cattle from different region and different farm of governorate include AL-dujail city, AL-dlwoeia city, AL-eshaki city, Balad city and Samara AL moqadsa, and from slaughter house of AL-dujail city.

All samples were collected from apparently healthy cattle before slaughtering and the volume of sample average from 20-25 gm. in spite of collected the tissue sample from internal organs from infected animals after slaughtering or death that suspected infect with Salmonella for histopathlogical study after confirm the results in laboratory.

Each sample was submitted to bacterial culture by using standard methods.

Culturing of specimens

Take out 1 g faeces from each sample for all species with a sterile wood spatula, put it into an Erlenmeyer flask etc. and add 9 ml buffered peptone water, Mix and Incubate at 37°C overnight (18-24 hours) then Transfer 1 ml of the pre-enrichment with a pipette to 10 ml.

Tetrathionate broth (Müller-Kaufmann). Incubate at 41.5°C ± 0.5°C overnight (18-24 hours) then Spread a 10 ìl loop full from the inoculated and incubated Tetrathionate broth on XLD and on SS agar plates and incubate at 37°C overnight (18-24 hours) and read the XLD plates and SS plates.Salmonella suspect colonies on XLD and SS agar onto non-selective media,

(nutrient agar) plates for biochemical confirmation of Salmonella (WHO b, 2010; Mezal *et al.,* 2016).

Histopathological study

Samples for histopathological examination was taked from infected animals (suspected infect with *Salmonella* or NTS) after death or slaughtered from internal organs (liver, intestine and mesenteric lymph nodes) and the tissues repair in a 10% solution of formaldehyde immediately after removal, in spite of collected fecal sample during infective stage and tissue sample after death or slaughter to isolation of bacterium (Atyabi *et al.,* 2012), after positive result and confirm this result by serological methods in Central Public Health Laboratories, the samples handling according to routinely work by (Luna, 1968). Two samples was collected for histopathological examination from infected cases.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant

compare between percentage (0.05 and 0.01 probability) in this study.

Results

Isolation of *S. typhimurium* from cattle

Nine isolates were isolated from the 105 samples of cattle from 5 region, AL-Dujail city, Balad city, AL-Dlwoeia city, AL-Eshaki city, and Samara AL Moqadsa, and from slaughter house of AL-dujail city and privet farms for detection of infected case. The results was recorded from male and female were 8.57 %, the details of the results explained in table(1) below. (Table 2) explaining the distribution of cattle isolates of *S. typhimurium* according to age in AL-dujail area, in Balad area as in (Table 3). (Table 4) showing the distribution of isolates of *S.typhimurium* nin AL-Dlwoeia area, and distribution in AL-Eshaki area table 5, and in table 6 appearing the isolates of *S.typhimurium* according to age of cattle in Samarra area.

Histopathological study

Histopathological section in one case of intestine of pregnant cow infected with *Salmonella typhimurium* showed necrotic with distorted villi infiltration of inflammatory cells with mucosa and submucosa which is edematous and focal ulceration and thickening of blood vessel wall (Fig. 1), sloughing of mucosa layer and thickening of submucosa that infiltrated with neutrophile and plasma cells (Fig.2). Thickening of blood vessel wall and presence of neutrophiles and lymphocytes (Fig. 3) in liver showed dilated of sinusois and hepatocytes appeared like cords with granulation of cytoplasm of hepatocytes (red raw) and infiltration of inflammatory cells like kupffer cells epatocytes appeared like cords with granulation of cytoplasm of hepatocytes and infiltration of inflammatory cells like kupffer cells (Fig. 4).

Discussion

Fecal samples were cultured on MacConkey and Eosine Methylene Blue agar isolation, Xylose Lysine Deoxycholate and Salmonella –Shigellaagar for *S. typhimurium* isolation as in (12). The results of our study in cattle showed low percentage of isolation 8.57% in both sex male and female ($n = 9\backslash105$) this may be attributed to the time when the samples were collected in the cold season on the other hand according to gender the study showed moral difference between male and female percentage 12.5% and 7.86% respectively, this results compatible with the results of (13) when he isolated 5% of *S. typhimurium* from 100 cattle fecal sample in north of Basra for both sex, also agreement with (14) who report that isolation rate of salmonella was 7% from

Table 1: Explaining the distribution of cattle isolates of *S.typhimurium* according to sex and region.

Region	No. of samples			Positive result			Percentage%		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
AL-dujail	5	20	25	1	2	3	20	10	12
Balad	2	18	20	0.00	1	1	0.00	5.55	5
AL-Dlwoeia	2	13	15	0.00	1	1	0.00	7.69	6.66
AL-Eshaki	3	17	20	0.00	1	1	0.00	5.88	5
Samarra	4	21	25	1	2	3	25	9.52	12
Total	16	89	105	2	7	9	12.5	7.86	8.57
Chi square value	—	—	—	—	—	—	7.653 **	2.319 NS	4.022 *
P- value	—	—	—	—	—	—	0.0074	0.092	0.0488

* (P<0.05), ** (P<0.01).

Table 2: Explaining the distribution of cattle isolates of *S.typhimurium* according to age in AL-dujail area.

Sex	Total male and female	3 years and below	Positive results	%	Above 3 years	Positive results	%
Male	5	2	0.00	0.00	3	1	33.33
Female	20	10	1	10	10	1	10
Total	25	12	1	8.33	13	2	15.38
Chi square value	—	—	—	4.68 *	—	—	8.502 **
P- value	—	—	—	0.035	—	—	0.0059

* (P<0.05), ** (P<0.01).

Table 3: Explaining the distribution of cattle isolates of *S.typhimurium* according to age in Balad area.

Sex	Total male and female	3 years and below	Positive results	%	Above 3 years	Positive results	%
Male	2	1	0.00	0.00	1	0.00	0.00
Female	18	8	1	12.5	10	0.00	0.00
Total	20	9	1	11.11	11	0.00	0.00
Chi square value	—	—	—	5.173 *	—	—	0.00 NS
P-value	—	—	—	0.0287	—	—	1.00

* (P<0.05).

Table 4: Explaning the distribution of cattle isolates of *S.typhimurium* according to age in AL-Dlwoeia area.

Sex	Total male and female	3 years and below	Positive results	%	Above 3 years	Positive results	%
Male	2	1	0.00	0.00	1	0.00	0.00
Female	13	5	0.00	0.00	8	1	12.5
Total	15	6	0.00	0.00	9	1	11.11
Chi square value	—	—	—	0.00 NS	—	—	5.173 *
P-value	—	—	—	1.00	—	—	0.0287

* (P<0.05).

100 samples of cattle in a study done in Babylon governorate Thirty six samples out of 1124 samples from cattle feces were isolated as positive for *S. typhimurium* were collected from cattle in Markazi provinces of Iran in a study was carried out by (15) this results relatively less than the results of our study may be due to differences between epidemiological causative and regional.

While (16) recorded low percentage of isolation 2% when he collected 250 fecal sample (5 positive results) and he noted that S. Dublin waspredominant serovar in Ireland that disagree with our results, and the researcher (17) has indicated in his study The type of *serovars* isolated from the samples of different animal species confirms the different source of contamination, making it even more

Table 5: Explaining the distribution of cattle isolates of *S.typhimurium* according to age in AL-Eshaki area.
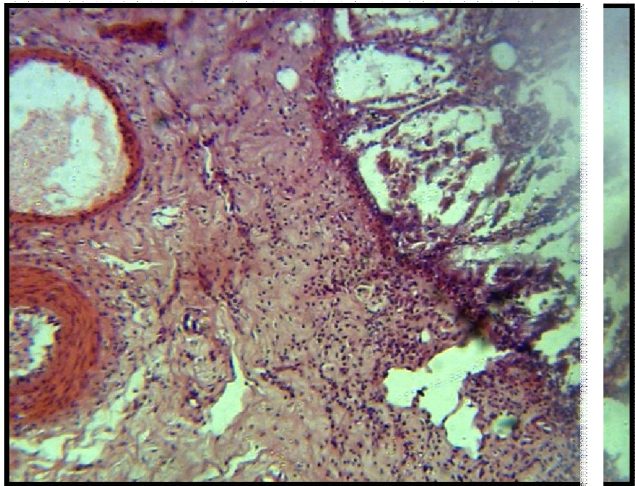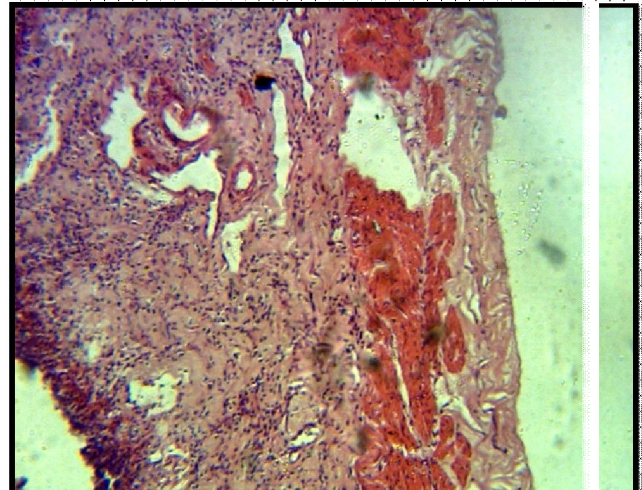
Sex	Total male and female	3 years and below	Positive results	%	Above 3 years	Positive results	%
Male	3	2	0.00	0.00	1	0.00	0.00
Female	17	8	1	12.5	9	0.00	0.00
Total	20	10	1	10	10	0.00	0.00
Chi square value	—	—	—	5.173 *	—	—	0.00 NS
P-value	—	—	—	0.0287	—	—	1.00

* ($P<0.05$).

Table 6: Explaining the distribution of cattle isolates of *S.typhimurium* according to age in Samarra area.

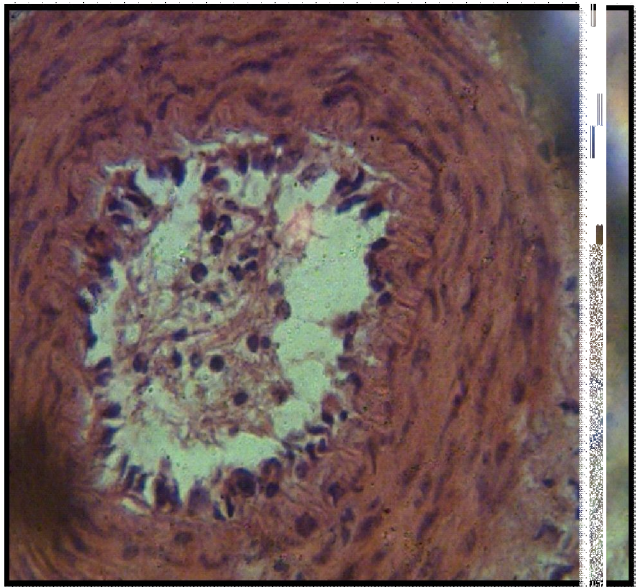| Sex	Total male and female	3 years and below	Positive results	%	Above 3 years	Positive results	%
Male	4	3	1	33.3	1	0.00	0.00
Female	21	9	0.00	0.00	12	2	16.66
Total	25	12	1	8.33	13	2	15.38
Chi square value	—	—	—	9.58 **	—	—	6.25 **
P-value	—	—	—	0.0002	—	—	0.0087

** ($P<0.01$).

Fig. 1: Histopathological section in one case of intestine of calf infected with *Salmonella typhimurium* showed necrotic with distorted villi infiltration of inflammatory cells with mucosa and submucosa which is edematous and focal ulceration (red raw) and thickening of blood vessel wall (blue raw) (H&E stainX100).

Fig. 2: Histopathological section in one case of intestine of calf infected with *Salmonella typhimurium* showed sloughing of mucosa layer (blue raw) and thickening of submucosa that infiltrated with neutrophile and plasma cells (red raw) (H&E stainX100).

interesting to study the different possible sources of contamination considering the Diversity of carrier individuals when he isolated A total of 2193 samples from different origins (feces of cattle, sheep, horses, pigs, dog, chickens and seagulls and chicken eggs) and the percentage of *S. typhimurium* in cattle was 6.25% which consistent with our study In south Africa *S. typhimurium* was considered the widest zoological distribution serotype when it was mentioned in a surveillance by (18) was conducted on Salmonel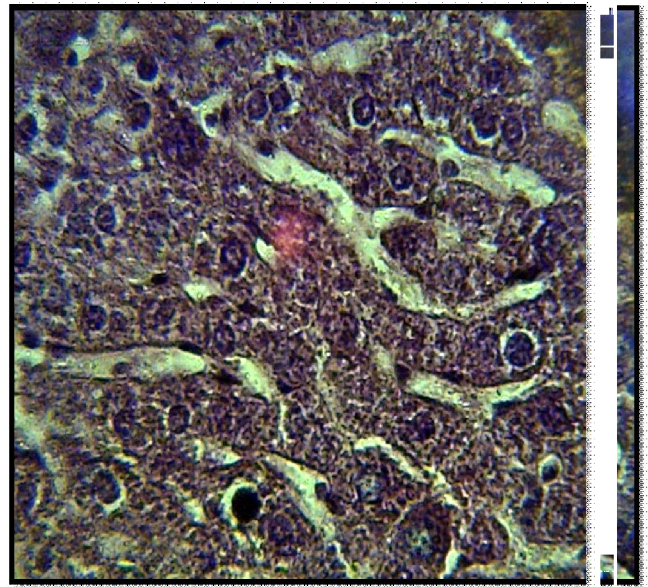la serotypes isolated from various animal species from 2007 to 2014, and the percentage in cattle was (14.6%) This finding is not unexpected because *S. Typhimurium* has been thought of as the most ubiquitous and broadest host-range serotype, as it is frequently associated with diseases in numerous host species, including humans, livestock, and this percentage of isolation higher rate than our study but we agree with his opinion about *S. typhimurium* as being the most common serotype, in contrast our study consistent with (19) when he got isolation ratio in salmonella 6.93% out

Fig. 3: Histopathological section in one case of intestine of calf infectedwith *Salmonella typhimurium* showed thickening of blood vessel wall and presence of neutrophiles and lymphocytes (red raw) (H&EX400).

Fig. 4: Histopathological section in one case of liver of calf infected with *Salmonella typhimurium* showed dilated of sinusois and hepatocytes appeared like cords (blue vraw) with granulation of cytoplasm of hepatocytes (red raw) and infiltration of inflammatory cells like macrophage cells (red raw) (H&EstainX400).

of 202 sample from cattle by a study in Ethiopia and he recorded that age and agroecology were a crucial factors related with the prevalence of Salmonellosis. Though, the overall prevalence reported by the current study is not considered to be high, but it could not be neglected because of its zoonotic importance. For this reasons, control and prevention of salmonellosis in live animals and implementation of risk reduction strategies should be implemented. *Salmonella typhimurium* in histopathplogical sections appeared necrotic with distorted villi infiltration of inflammatory cells with mucosa and submucosa which is edematous and focal ulceration and thickening of blood vessel wall, sloughing of mucosa layer and thickening of submucosa that infiltrated with neutrophile and plasma cells in case of pregnant cow and cause dying through three days may be due to acute infection with virulence NTS from one side and also exposed to stress factor that may be was the main factor of acute and re infection with virulence NTS include pregnant state, transporting from one area to another and may infected with parasite that cause immunosuppressant. *S. typhimurium* is a major cause of calf morbidity and mortality in the United States and in Europe (20). (21) experimentally referred o the microorganisms *Salmonella typhimurium* have the ability to infect ileum and penetrate to other internal organs. Furthermore, the infection in calves closely resembles illness caused by *S. typhimurium* in humans and can therefore serve as amodel to study diarrheal disease. Calves infected experimentally with *S. typhimurium* develop diarrhea within 48 h (22) invade the intestinal epithelium in the terminal ileum, resulting in exfoliation of epithelial cells and stunting of villi (23).

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