

## DISTRIBUTION OF *SALMONELLA* SPECIES IN BUFFALOES IN SOME MIDDLE GOVERNORATES OF IRAQ

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### ABSTRACT

This study was planned to isolate and type *Salmonella* from buffalo in three middle governorates in Iraq. One hundred fifty milk samples were collected from 150 lactating buffaloes in the field, and in the slaughter house (150) fecal samples were collected at slaughter time and (900) samples were collected post slaughtering: 150 samples from each organ: gall bladder, mesenteric lymph nodes, spleen, liver, small intestine and cecum.

Diagnostic study depended upon the morphological and cultural properties of the isolates on some selective media like Brilliant Green, XLD, SS agar and Hicrome rajhans medium, modified (*Salmonella* agar modified) were used in addition to different biochemical tests, API-20E and KB 003 Hi25<sup>TM</sup> Enterobacteriaceae Identification Kit, latex test and serotyping of isolates.

Twenty-two isolates were obtained from the fecal samples and organs; these isolates belonged to three serotypes (*Salmonella anatum* (68.18%), *S.muenchen* (18.18%) and *S. enteritidis* (13.64%), while no isolate was obtained from milk samples.

In animals from slaughter houses, the percentage of infection varied in examined organs and feces: in bile duct and liver, the percentage was 3.33% each; in mesenteric lymph nodes, 2.67%; in spleen and cecum, 2% each; and in feces, 1.33%.

The antibiotic susceptibility pattern of *Salmonella* against 15 antimicrobial drugs revealed that all isolates were resistant (100%) to chloramphenicol, suphamethoxazole, erythromycin, cloxacillin, and tetracycline, and all isolates were sensitive to ampicillin and trimethoprim while neomycin, gentamycin, cefixime, ciprofloxacin, kanamycin and streptomycin gave intermediate results.

**Keywords:** *Salmonella*, buffaloes, serotyping of *Salmonella*, Iraq

### INTRODUCTION

Buffalo raising is a major contributor to the agriculture and livestock industry in many Asian countries through the production of good quality meat and farmyard manure (Singh, 2010). The buffalo is also recognized as the world second most important milk producing species (Bhatti *et al.*, 2009). Dairy buffaloes are also called the 'black gold' of South Asia, where 95% of the world's buffalo milk is produced (Javaid *et al.*, 2009). Therefore, the pathogens either causing disease in buffaloes and their progeny or transmitted through their production are important because they affect milk production and overall livestock production.

Salmonellosis is associated with medium to

severe morbidity and even mortality in farm animals, representing a major economic productivity loss in the food and animal industries (Malkawi et al., 2004). *Salmonella* has been widely reported in buffaloes (Hassanain et al., 2010; Khan et al., 2009; Ribeiro, 2000; Abdulwahid and Raheem 1981), and infected animals may shed the organism in their feces without showing any clinical signs of disease (Fardsanei et al., 2010). Therefore, buffaloes may carry this organism undetected into an abattoir at the time of slaughter.

This study aimed at determining the distribution of *Salmonella* in different organs, feces, and milk samples of buffaloes in three governorates in Iraq and at determining the sensitivity of the obtained isolates to different antimicrobials.

## MATERIALS AND METHODS

The study was performed on 300 buffaloes distributed as follows: 150 dairy buffaloes in the field and 150 buffaloes in slaughter houses.

Information about animals concerning age, sex, and any signs of diarrhea were recorded. One hundred fifty milk samples were collected from lactating buffaloes in the field, and in the slaughter house 150 fecal samples were collected at slaughter time and (900) samples postslaughtering samples were collected, distributed as follows: 150 samples of gall bladder, 150 samples of mesenteric lymph nodes, 150 samples of spleen, 150 samples of liver, 150 samples of small intestine and 150 samples of cecum.

The isolation and biochemical identification of *Salmonella* was carried out according to standard laboratory methods (Quinn et al., 2004). Each sample was transferred into tetrathionate broth for *Salmonella* enrichment before streaking onto

Brilliant Green agar, Xylose-Lysine Deoxycholate agar, *Salmonella-Shigella* agar and Hicrome rajhans modified *Salmonella* agar and incubated aerobically at 37°C for 24 h. *Salmonella* suspected colonies were identified by Gram staining, in addition to different biochemical tests: motility, triple sugar iron agar, indole, methyl red and citrate utilization tests, API-20E and the use of KB 003 Hi25<sup>TM</sup>-Enterobacteriaceae Identification Kit, which is a standardized colorimetric identification system utilizing thirteen conventional biochemical tests and eleven carbohydrate utilization tests based on the principle of pH change and substrate utilization (Thangamaal et al., 2009).

Then, the isolates were grouped by the Wellcolex color latex test for *Salmonella* (Rohner et al., 1992). Description of kit contents (Figure 1).

1. Latex Reagent 1: one drop of gray-brown suspension of polystyrene latex particles in buffer containing 0.05% Bronidox® preservation. The latex particles are coated with rabbit antibody with the following specificity:

Red latex *Salmonella* group B; Blue latex *S.* group C; Green latex; *S.* group D.

2. Latex Reagent 2: one drop of gray-brown suspension of polystyrene latex particles in buffer containing 0.05% Bronidox® preservation. The latex particles are coated with rabbit antibody with the following specificity:

Red latex Vi; Blue latex *S.* group E and G; Green latex *S.* group A.

3. Red positive control: Killed bacterial suspension of organisms with *Salmonella* group B and Vi antigens containing 0.05% Bridox® and 0.5% formalin as preservative.

4. Blue positive control: Killed bacterial suspension of organisms with *Salmonella* group C and E antigens containing 0.05% Bridox® and 0.5%



Figure 1. Wellcolex color latex test for *Salmonella*.



Figure 2. Morphology and color of *Salmonella* colonies in different media. (A- Black arrow → colonies on Hicrome rajhans modified medium, B- green arrow → colonies on Brilliant green agar C- yellow arrow → colonies on XLD, D- blue arrow → colonies on SS agar).

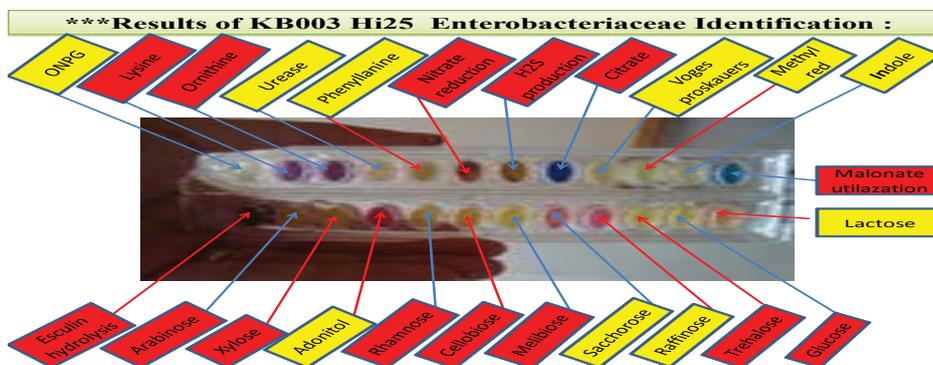


Figure 3. Showing results to KB003 Hi25 Enterobacteriaceae.

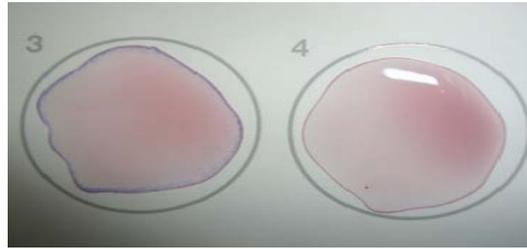


Figure 4. *Salmonella* serogroup : E (blue agglutination- pink background with Reagent 2).

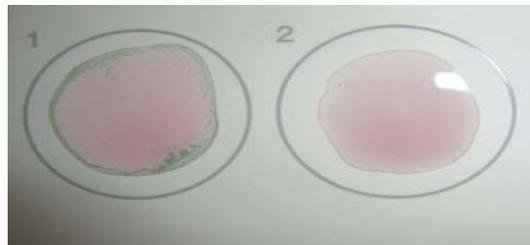


Figure 5. *Salmonella* serogroup D : (green agglutination-pink background) with reagent 1.

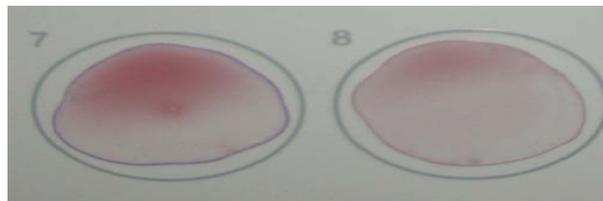


Figure 6. *Salmonella* serogroup G (blue agglutination-pink background) with reagent 1.

formalin as preservative.

5. Green positive control: Killed bacterial suspension of organisms with *Salmonella* group A and D antigens containing 0.05% Bridox® and 0.5% formalin as preservative.

Finally, serotypings of *Salmonella* isolates were confirmed in the Central Public Health Laboratories by using specific antisera.

Antibiotic susceptibility tests for *Salmonella* isolates were performed according to the Kirby Bauer method (Bauer *et al.*, 1996). Mueller Hinton agar was used as growth medium for standard disc diffusion test and growth was spread on plates with the help of a sterilized cotton swab to form a smooth bacterial lawn. The discs were placed on to the agar surface using sterile forceps. Each disc was gently pressed with the point of sterile forceps to ensure complete contact with the agar surface.

Plates were incubated overnight at 37°C. Characterization of strains as sensitive or resistant was based on the size of the inhibition zone around the disc compared with the interpretation standards provided by the manufacturers. The antimicrobial drugs used were ampicillin, bacitracin, chloramphenicol, erythromycin, gentamycin, kanamycin, novobiocin, penicillin, spectinomycin, streptomycin, tetracycline and trimethoprim.

## RESULTS

### Isolation and identification of *Salmonella* spp.

Isolation and identification of *Salmonella* were confirmed in different media as showed in Figure 2. The cultural characteristics showed different colonies, On *Salmonella* Shigella agar, the colonies appear as small pale, rounded with black center, on Xylose-Lysine Deoxycholate agar, they showed slightly transparent zone of reddish and

black center, on Brilliant green agar the colonies appeared gray reddish/pink slightly convex and on Hicrome rajhans modified medium the colonies appeared as pink in color.

The results of biochemical tests by Api and KB003 Hi25 Enterobacteriaceae Identification system showed that this bacterium was positive for: Lysine utilization, Ornithine utilization, Nitrate reduction, H<sub>2</sub> S production, Citrate utilization, Methyl red, Arabinose, Xylose, Rhamnose, Melibiose, Glucose; and negative for: ONPG, Urease, phenylalanine Deamination, Voges Proskauer's, Indole, Malonate utilization, Esculin hydrolysis, Adonitol, Cellobiose, Saccharose, Raffinose, Trehalose, Lactose, Oxidase (Figure 3).

The results of serogrouping of *Salmonella* isolate according to serogrouping Wellcolex Color *Salmonella* showed that and 4 isolates gave blue agglutination-pink background with reagent 2 [it belong to group E, (Figure 4)]; 3 isolates gave [green agglutination- pink background with reagent 1 [it belongs to serogroup D, (Figure 5)] and 15 isolates gave blue agglutination-pink background with reagent 1 [it belong to group G, (Figure 6)]. Table 1 showed of *Salmonella* grouping and serotyping.

### Percentage of infection with *Salmonella* in buffaloes

Microbiological examination of samples that had been collected from slaughter houses (organs and feces ) revealed that seven out of 150 were positive for *Salmonella*. The milk samples of buffalo in the field revealed negative results so that the statistic analysis showed significant differences at  $p > 0.05$  between animals at the slaughter house and animals in the field.

### Percentage of infection with *Salmonella* species

### **in buffaloes in the slaughter house**

The results of serotyping of *Salmonella* spp. from (1050) samples at the slaughter house were that 22 isolates of three different serotypes were recognized according to the Central Public Health Laboratories. These serotypes were *Salmonella anatum* (68.18%), *Salmonella muenchen* (18.18%) and *Salmonella enteritidis* (13.64%). The statistical analysis showed significant differences at  $p > 0.05$  between *Salmonella anatum* and the others.

### **Clinical signs**

Out of 41 animals, three animals showed different clinical signs: diarrhea (1), respiratory signs (1) and more than one symptom (1) were affected with *Salmonella*, while four animals out of 109 examined that gave positive results for *Salmonella* did not show clinical signs (Table 2). In the field, all animals with acute or chronic mastitis and without clinical signs gave negative results for *Salmonella* infection.

### **Percentage of *Salmonella* isolated from different samples**

*Salmonella* was isolated from different samples with different percentages except samples of small intestine and milk. The highest percentages appeared in the gall bladder (3.33%) and the liver (3.33%) followed by mesenteric lymph nodes (2.67%). Statistical analysis showed significant difference at ( $p < 0.05$ ) (Table 3).

### **Distribution of *Salmonella* isolated according to age**

According to age of buffaloes at the slaughter house, the results show that *Salmonella* was highest (6.67%) in the 1.5 - 3 year age group and lower (3.23%) in animals more than 3 years old. Statistically no significant difference between

age groups of buffaloes was found as shown in Figure 7.

### **Distribution of *Salmonella* species according to sex**

Thirteen isolates from 97 samples were recorded in males and nine isolates from 53 were recorded in females. Statistically, there was no significant difference between males and females at  $p \geq 0.05$ .

### **Distribution of *Salmonella* spp according to month of year**

Figure 8 shows the distribution of *Salmonella* spp. according to month of year. No isolates appeared in November and December, one affected animal (1 isolate) was found in January, but in February, three affected animals (12 isolates) were found, two affected animals (6 isolates) were found in March, and one affected animal (3 isolates) was found in April.

### **Results of antimicrobial sensitivity tests**

The antibiotic susceptibility pattern of *Salmonella* against the antimicrobial disc revealed that all isolates were resistant (100%) to chloramphenicol, sulphamethoxazole, erythromycin, cloxacillin, and tetracycline. All isolates were sensitive to ampicillin and trimethoprim.

## **DISCUSSION**

The results of the present study showed that *Salmonella* were isolated at a percentage of 2.33% buffaloes, and this is in agreement with the study of Hassanain 2008 which recorded a percentage of 2.16% of *Salmonella* infection in buffaloes in

Egypt, and with a study of Al-Nakshabandy (2001) in buffaloes, which found that the overall ratio of infection with *Salmonella* in Mosul city in Iraq was 2.23%. while Sen *et al.* (1988) recorded the low percentage 1.60% of *Salmonella* from rectal swabs of buffalo in Bangladesh. Phillips *et al.* (2008) isolated *Salmonella* from 1.1% of ground buffalo beef samples.

Other studies have also shown different results, Hassanain *et al.* (2010) found a high percentage (11.11%) of *Salmonella* in buffaloes in Egypt. Also Khan *et al.* (2009) found higher percentage (16.3%) of *Salmonella* during study of diarrhea in buffalo calves in Pakistan .

Boonmar *et al.* (2008) isolated *Salmonella* from fecal samples of buffaloes in Japan at a percentage of 8%. Sharma *et al.* (1989) found 16.53% of meat samples were positive to *Salmonella* in Indian buffaloes.

No significant difference between *Salmonella* infection in the three governorates may explained by their being in the same geographical region and having the same climate. Also. there was easy transmission of animals between them. No study is available concerning the distribution of *Salmonella* in buffaloes at slaughterhouses in all governorates in Iraq.

These results resemble those of other studies. In Pakistan, Ali *et al.* (2008) recorded negative results for *Salmonella species* in 200 mastitis quarters in buffaloes. Also, negative results for *Salmonella* in Iraqi buffaloes were recorded by Abdul Razak. (1982).

Our study is compatible with many studies which showed that *Salmonella* was not isolated from milk samples, such as Coroian *et al.* (2010) in Romanian buffaloes ; Khan *et al.* (2009) in Pakistan ; Ali *et al.* (2008) in Pakistan, and Moroni *et al.* (2006) in northern Italy.

In contrast, a study in Pakistan by Iqbal *et al.* (2004) showed isolation of *Salmonella* at a percentage of 2.41% in milk samples of lactating dairy buffaloes .

The results of present study were in agreement with those of Gunasegaran, 2011 who found that *Salmonella* was resistant to tetracycline and chloramphenicol and sensitive to kanamycin. Also, Singh, *et al.* (2010) recorded that 46 strains of *Salmonella enteric.* were resistant to streptomycin and kanamycin but disagreed about resistance to gentamicin and amoxicillin.

The present study showed that the *Salmonella anatum* and *Salmonella enteritidis* were resistant to seven antimicrobials and *Salmonella meunchen* was resistant to eight antimicrobials , while a study by Boonmar *et al.* (2008) recorded that the buffalo isolates were susceptible to most of antimicrobials (tetracycline, streptomycin, ampicillin, sulfamethoxazole-trimethoprim, chloramphenicol, amoxicillin-clavulanic acid and nalidixic acid) and all isolates showed sensitivity to cefotaxime, norfloxacin and ciprofloxacin.

In the present study, some animals showed different clinical signs of *Salmonellosis*, and these signs were similar to those recorded by Santana *et al.* (2008) in their study on experimental infection with *Salmonella dublin* in buffalo calves, which showed the main clinical signs, i.e., diarrhea, fever, respiratory signs and dehydration while other animals without clinical signs but gave positive results for *Salmonella* infection. This revealed a number of carrier animals without clinical signs, confirming Nabbut and Al-nakhlil (1982), Galland *et al.* (2000), and Radke *et al.* (2002) who found that animals which had recovered from *Salmonellosis* may continue shedding of *Salmonella* microorganisms from 2-12 weeks post infection, whereas shedding of *Salmonella* microorganisms from animals from

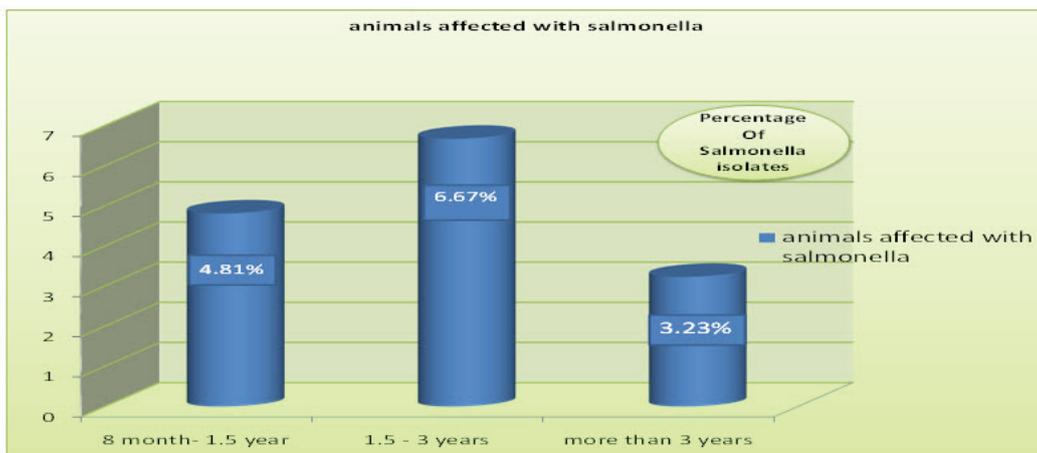


Figure 7. Distribution of *Salmonella* isolated according to age.



Figure 8. The distribution of *Salmonella* spp. according to month.

Table 1. Results of *Salmonella* grouping and serotyping.

Total No. of <i>Salmonella</i> isolates	No. of isolates	No. isolate	Serotyping at center of <i>Salmonella</i>
22	15	Group E	<i>Salmonella anatum</i> (68.18%)
	3	Group D	<i>Salmonella enteritidis</i> (13.64%)
	4	Group G	<i>Salmonella muenchen</i> (18.18%)

Statistical results showed significant difference at  $p < 0.05$ .

Table 2. Distribution of affected animals according to clinical signs before slaughtering.

Clinical signs	No. of animals	No. of affected animals with <i>Salmonella</i>
With clinical signs	41	3 animals (diarrhea(1), respiratory signs (1) and more than one signs (1))
Without clinical signs	109	4 animals

Table 3. Percentage of *Salmonella* isolated from different samples.

Collection of samples	Sample	No. of examined sample	No. of positive sample	No. of <i>Salmonella</i> spp.
Organ and feces at slaughter house	Gall bladder	150	5	4 <i>S. anatum</i> , 1 <i>S. munchen</i>
	Mesenteric lymph node	150	4	3 <i>S. anatum</i> 1 <i>S. munchen</i>
	Spleen	150	3	2 <i>S. anatum</i> 1 <i>S. munchen</i>
	Liver	150	4	3 <i>S. anatum</i> 1 <i>S. munchen</i> 1 <i>S. enteritidis</i>
	Cecum	150	3	2 <i>S. anatum</i> 1 <i>S. munchen</i>
	Small intestine	150	0	0
	Feces	150	2	1 <i>S. anatum</i> 1 <i>S. enteritidis</i>
	Total at slaughter house	1050	22	22
Field	Milk	150		0
	Total	1200		22

Table 4. Antibiotic susceptibility pattern of *Salmonella*.

No.	Antibiotics used	Numbers of <i>Salmonella</i> isolates					
		R	%	I	%	S	%
1	Chloramphenicol	22	100	0	0	0	0
2	Neomycin	2	9.1	15	68.2	5	22.7
3	Suphamethoxazol	22	100	0	0	0	0
4	Gentamicin	21	95.5	0	0	1	4.5
5	Cefixime	18	81.9	4	18.2	0	0
6	Erythromycin	22	100	0	0	0	0
7	Ciprofloxacin	0	0	1	4.5	21	95.5
8	Kanamycin	1	4.5	11	50	10	45.5
9	Amoxicillin	21	95.5	1	4.5	0	0
10	Streptomycin	0	0	1	4.5	21	95.5
11	Tetracyclin	22	100	0	0	0	0
12	Amicacin	0	0	0	0	22	100
13	Trimethoprim	0	0	0	0	22	100
14	Cloxacillin	22	100	0	0	0	0
15	Nitrofurantoin	1	4.5	2	9.1	18	81.8

which clinical signs had disappeared may be found with chronic infection of *Salmonellosis*. Carrier animals may also be clinical cases if resistance is lowered by environmental stress or inter current infection.

In the present study, data demonstrated that 22 *Salmonella* isolates were isolated from 1050 samples, constituting about 2.09%, and that three different species were found. This results is consistent with Singh *et al.* (2010) who recorded different species in buffaloes (*S. anatum*, 13; *S. weltevreden*, 13; *S. rostock*, 6; *S. typhimurium*, 5; *S. gallinarum*, 5; *S. stockholm*, 1; *S. dublin*, 1; and *S. orion*, 2) and with two studies: Hassanain *et al.* (2008) and Hassanain (2010) who found two species in buffaloes (*S. enteritidis* and *S. typhimurium*).

In India, Sharma *et al.* (1989) recorded different species in buffaloes: *S. anatum*, *S. bareilly*, *S. stanley*, *S. Weltevreden*, *S. newport*, *S. saintpaul*, *S. typhimurium*, *S. agona*, *S. chester*, and *S. senftenberq*, while Boonmar *et al.* (2008) in Japan found two species only: *S. derby* and *S. javiana*.

The results of the present study resemble a study in Pakistan by Ali *et al.* (2008) which recorded no infection for *Salmonella* in 200 mastitic quarters in buffaloes, and also a study by Abdul Razak (1982) in Iraqi buffaloes which showed no infection in mastitic milk in buffaloes. Also, many previous studies reported similar findings.

In the present study, the data demonstrated a higher percentage of *Salmonella* isolates from the gall bladder and the liver, whereas all of the *Salmonella* serotypes (*S. anatum*, *S. enteritidis*, *S. muenchen*) were isolated from the gall bladder and the liver at a percentage of 40% from the total of *Salmonella* isolates in this study followed by isolates from the mesenteric lymph nodes, the spleen and the cecum.

Our results also showed that the higher percentage of *Salmonella* isolated from organs as compared with feces. The reason for this might be by that the body does not shed *Salmonella* microorganisms continuously in feces: particularly, carrier animals with *Salmonella* shed microorganisms intermittently. This is in agreement with the study of Molla *et al.* (2002) which reported a high percentage of *Salmonella* isolates (4.2%) in organs (mesenteric lymph node) and a low percentage in feces (1.9%). These results resemble those of Poernomo *et al.* (1986) who found that the percentage of *Salmonella* isolates from fecal samples (1.7%) was lower compared with the percentage of *Salmonella* isolates from organs (3.12%).

Our study revealed a difference in the distribution of *Salmonella* in organs and feces. This indicates that an animal may be a carrier of *Salmonella* in its organs or actively excrete *Salmonella* in its feces. This is in agreement with a study of Singh *et al.* (2010) who mentioned that if the animal was infected with *Salmonella* organisms, it may become a clinical case or an active or latent carrier. In the active carrier cases, the organisms are localized in the intestine and gall bladder, from whence they are excreted with the feces, contaminating the environment and posing to a threat to susceptible hosts. In latent carrier cases, the organisms are localized in the lymph nodes, liver, spleen and tonsils, but the organisms are not excreted with the feces.

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