

# **RESEARCH ARTICLE**

# CLINICAL AND HEMATOLOGICAL STUDY OF EXPERIMENTAL SALMONELLOSIS IN AWASSI LAMBS (OVISARIES).

#### Roaa A.Hussein<sup>1</sup>, Ibrahim Abdul-H. Al-Zubaidy<sup>1</sup>, Saad S. Farkhry<sup>3</sup> and Farqad F. Abdul hameed<sup>2</sup>.

.....

- 1. Department of Veterinary Internal and Preventive Medicine, University of Baghdad.
- 2. Food contamination Research Center. Environment and Water Directorate. Ministry of Science and Technology.

Manuscript Info

# Abstract

Manuscript History

Received: 12 July 2016 Final Accepted: 19 August 2016 Published: September 2016

Key words:-Clinical, hematology, salmonellosis, Awassi lambs and Salmonellatyphimurium. This study was carried out to study the clinical, hematological changes in Awassi lambs*Ovisaries* experimentally infected with *Salmonella typhimurium*.Ten (10) lambs were included in this study which divided into two groups. The first group inoculated have five lambs (control group) while the second group were inoculated orally with 10 mL of trypticase soya broth which containing  $1 \times 10^{11}$  CFU /mL of *Salmonella typhimurium*(infected group). All animals were observed daily,infected group and control group investigated the clinical, hematological parameters and the results revealed that, the lambs experimentally infected with *Salmonella typhimurium*showed gastrointestinal forms of the disease. The results also revealed that, there were severe hematological and clinical changes in lambs experimentally infected with *Salmonella typhimurium*.

Copy Right, IJAR, 2016,. All rights reserved.

.....

#### Introduction:-

Salmonella is an important pathogen to the food industry and has been frequently identified as the etiological agent of foodborne outbreaks (Tauxe, 1999 and Zhao *et al.*, 2001).Salmonella Typhimurium is the most common Salmonella serovar in sheep. Infection with Salmonella Typhimurium often results in both enteric and systemic symptoms.Salmonellosis of sheep is an infectious bacterial disease causing illness and death. It results from proliferation of Salmonella bacteria in the gastrointestinal tract and other organs (Baker*et al.*, 2007).Salmonella infections represent an economic and potential public health risk on sheep and goat farms. Animals with clinical signs of gastro-intestinal disease or septicemia may pose the highest risk for humans,the contacts with small ruminants pose a potential health risk to occupationally exposed subpopulations as well as the general public, but the risk depends strongly on the serotype involved (Foneand Barker 1994; Ohl and miller, 2001), Include domestic sheep Ovisaries, which known from 10000 years ago at south west of asia (Guo et al., 2005).

## Material and Methods:-

Ten healthy lambs about 2.5-4 months of age and with body weight range between 6-8 kg B.W were used to perform the experiment of the present study. Animals were checked for clinical salmonellosis. Animal group that show clinical enteropathogenic form of salmonellosis with characteristic signs of diarrhea and other clinical feature was depended in this study.

The animals included in this study were divided into two groups. **Control group:-** five animals of this group.

**Infected group:-** five animals of this group were inoculated orally with of trypticase Soya broth containing  $1 \times 10^{11}$  cfu CFU of *Salmonella typhimurim*per ml.

#### The following parameters have been studied

Physiological changes:- temperature, heart rate and respiration rate at the4, 8,12,16,20 day of studies.

**Hematologicalchanges:-** Determination of RBCs,Hb, PCV, WBCs and DLC weekly was estimated according to Coles (1986).

#### **Results:-**

The infected group of all animals, show signs of illness represented with fever, congested mucus membrane of the eye with engorged capillaries and showed depression, moderate dehydration and respiratory distress represented with head, neck extension toward rapid respiration and nasal discharge ,increase of heart rate in addition animals show difficult ability to move and even recumbence.

With regard to the general clinical signs of the infected group, show significant difference at ( $p \le 0.05$ ) for almost all clinical parameters. The most obvious alteration in clinical parameter occur in eighth day and twelfth day of infected group, this result convert gradually toward normal in day sixteen latter.

Also, the result of hematological values of lamb groups in table (2) mean  $\pm$  SE; of RBCs (10<sup>6</sup>/ml) and Hb (g/dl) associated with significant decrease (p $\leq$ 0.05) in the infected group compared to control group. As well as the pcv(%)associated with significant increase (p $\leq$ 0.05) in the infected group compared to control group.

The result in table (3) obtained the mean  $\pm$ SE for total and absolute differential leukocyte counts of lamb groups were as follows; WBCs (10<sup>3</sup>/ml) count of theinfected group compared to control group, moreover, the result registered significantly higher (p≤0.05) in the absolute differential leukocyte counts in the neutrophils, esinophiles and monocyte in the infected group compared to control group.

Parameter	Temperature rate <sup>o</sup> C	Heart rate/minute	Respiratoryrate/minute		
Groups					
Control group 4 <sup>th</sup> day	$0.08B \pm 39.2$	1.77C ±72.6	0.63B ±26		
Infected group 4 <sup>th</sup> day	$0.08A \pm 41.44$	3.10A±91.8	$1.16A \pm 38.4$		
Control group 8 <sup>th</sup> day	$0.08B \pm 39.17$	2.38B±71	0.5B±27.5		
Infectedgroup 8th day	0.02A ±41.37	4.08A±112	$0.95A \pm 40.5$		
Control group 12 <sup>th</sup> day	0.07B±39.2	3.30B ±71.5	0.95B±26.5		
Infected group 12 <sup>th</sup> day	0.12A±41.3	$6.62A \pm 106.75$	1.89A ±37.5		
Control group 16 <sup>th</sup> day	$0.13B \pm 39.06$	$1.76B \pm 74.66$	1C±24		
Infected group 16 <sup>th</sup> day	0.03A ±41.13	2.4A±92.66	1.5A ±34		
Control group20 <sup>th</sup> day	0.05B±39.2	$3.71B \pm 74.66$	1B±27		
Infected group 20 <sup>th</sup> day	0.06A±40.73	2.9A ±88.66	$1.33A \pm 30.66$		
$\mathbf{D}^{(0)}_{1}$					

#### **Table 1:-** The clinical values of lambsgroups in the 4, 8,12,16,20 day mean<u>+</u>SE.

Different letters horizontally mean significantly different at ( $P \le 0.05$ ).

Table	2:-	the hemato	ological	values (	of lamb	group; mean ±	⊦SE.
-------	-----	------------	----------	----------	---------	---------------	------

Parameter/ Groups	RBCs( $\times$ 10 <sup>6</sup> / $\mu$ L)	Hb (g/dl)	Pcv (%)
control group	11.5 ±0.26A	17.7 ±0.34A	33.1 ±0.63C
infected group	$6.8 \pm 0.25 \mathrm{C}$	$12.1\pm0.28B$	56.1±0.29A
5100 1 10			

Different letters mean significantly different at ( $P \le 0.05$ ).

		2			
Parameter Groups	WBCs $(\times 10^3/ \mu L)$	Lymphocytes $(\times 10^3/ \text{ µL})$	Neutrophiles $(\times 10^3 / \text{ u L})$	Monocytes $(\times 10^3 / \mu L)$	Esinophiles $(\times 10^3 / \mu L)$
control group	10.0±0.17C	7.3±0.15A	2.2±0.18C	0.19±0.01B	0.25± 0.01B
infected group	17.3±0.24A	9.8±0.17A	$6.8 \pm 0.13 \text{ B}$	0.30±0.01A	$0.32 \pm 0.02 A$
$D^{1}(0) = (1 + 1) + (1 $					

Table 3:- The total and absolute differential leukocyte counts of lamb group; mean ±SE.

Different letters mean significantly different at ( $P \le 0.05$ ).

## **Discussion:-**

Diarrhea showed in infected group was similar infrequency and circumstances with that of other studies that indicate a frequency and presence of blood and mucous fecal consistency was watery especially in first seventy two hours and then lightly spot, this picture is completely agree with all studies on salmonella enteritis these sings accompanied with extensive systemic involvement summarized by fever, dysentery, tensmus and putrid odor of feces(Radostitis 2007). Commonly *salmonella* is the major enteropathogenic organism that present in fecal samples of diarrheal animal (Farid et al., 1987). Our results revealed high concentration of salmonella organism in infected group due to localization of this organism in intestinal epithelium leading to bacterial enteritis. All animals show clinical signs of diarrhea where infected with salmonellosis due to impaired absorption, damage of intestinal epithelium which can be explained by the effect of enter toxins produced by *salmonella* pathogens in the small intestine (Aly et al., 1996). In our results animal show depression, dehydration and abdominal pain these signs was agree with (Ansaruzzaman *et al.*,2000 and Callaway et al., 2005).

The appearance of fever due to salmonellosis in infected group occurs due to production of low molecular weight protein (pyrogen) which resemble interlukn I and leukocytic endogenous mediator, this released from phagocyte cells in response to bacterial toxins ,in infected group high temperature continue to increase due to stages of bacteria occurs within twenty four hours of infection (Rosallie et al., 1985).Respiratory involvement reported in this study include shallow rapid respiration which may indicate sings of pneumonia. Lacremation, depression, dullness, congestion mucous membrane were also seen and also agree with studies under go on *salmonella* infection in sheep (Radostitis 2007).

Hematological picture shows significance decrease in RBCs count and Hb(g/dl)of infected group with significant increase in PCV(%), RBCs count decrease obviously due to losing of blood from intestinal mucosa this combinated by high losing of iron and blood protein which lead to decrease in Hb(g/dl)concentration to gather with decreasing albumin production by liver. Dehydration is the most possible cause of increasing PCV value (Mona et al.,2010). The accompanied signs showed in animal of this group where resulting from enter toxin produced by this bacteria(Aly et al.,1996) moreover signs related to anemia and disturbance in blood cell count relate to the effect of these enterotoxines with the direct bacterial effect in disturbance of absorption of tries elements through the damage of intestinal epithelium significant decrease in hemoglobin level indicate anemia which may be due to low ability of absorption of iron and copper with decrease of albumin synthesis by the liver (Mona et al.,2010).

Results of this study include many alteration in the type of WBCs count numbers in infected group the obvious alteration was neutropenia these mechanisms include the effect of entertoxin on heamobiotic precursor cells, infection of endothelial cells (Kramer*et al.*, 2001 and Balk. 2002). Our increasing the neutrophils adhering to endothelium of blood vessels in intestinal tract and other internal organs (Thomas, 2016). This supported by the facts that neutrophils are the professional phagocytes in the body (Gill et al., 2012) and *salmonella typhimurium* infection make an acute inflammatory sings characterized locally by massive infiltration of neutrophiles and rapid neutrophiles attraction from blood to local infection inside the body prevent *salmonella* spreading (Santos et al., 2002) and (Cedric et al., 2004), were as lymphocytes attracted too as aresponse of TH1 cytokine in the intestine and spleen this result maintiond also by (Bai et al., 2014). But as a result of decrease number of nutorophiles there was a false increase in lymphocytes in circulating blood.

## **References:-**

- 1. Aly, A.O.; Zamzam, H.A. Kohilo, KH and El-Sheilch, A. R. (1996): "Some studies on clinical, haematological and biochemical changes in diarrheic neonatal buffaloe-calves with reference to hygienic condition". AssiutVet... Med. J. 35(69): 91-101.
- Bai, S. P.; Huang, Y.; Luo, Y. H.; Wang, L. L.; Ding, X. M.; Wang, J. P.; Zeng, Q. F.and Zhang, K. Y. (2014). Alteration in lymphocytes response cytokines and chemokine profiles in laying hens infected with salmonella typhimurium. Vet. Immunol. and Immunopath. 160(3–4): 235–243.
- 3. Baker MG, Thornley CN, Lopez LD, Garrett NK and Nicol CM. (2007). A recurring salmonellosis epidemic in New Zealand linked to contact with sheep. Epidemiol Infect. 135(1):76–83.
- 4. Callaway TR, Keen JE, Edrington TS, Baumgard LH. (2005).Fecal prevalence and diversity of Salmonella species in lactating dairy cattle in four states. J Dairy Sci; 88:3603-3608.
- 5. Cedric, C.; Dipshika, C.andMichae, IH. (2004). Role of neutrophils in murine salmonellosis. Infect immun.; 72(1):468-477.
- 6. Farid, A. F.; Nashed, S. A. and Marcell, K.S. (1987): "Salmonellosis in bufaloe- calves in Upper Egypt". J. Egypt. Vet. Med. Ass. 47(182): 153-160.
- 7. Fone DL and Barker RM,(1994). Associations between human and farm animal infections with *Salmonella* Typhimurium DT104 in Herefordshire. Commun Dis Rep CDR Rev.; 4(11):R136–140.
- Gill, N., Ferreira, R.B.R., Antunes, L.C.M., Willing, B.P., Al-Zahrani, S.F., Hartmann, M., Finlay, B. B.(2012). Neutrophil Elastase Alters the Murine Gut Microbiota Resulting in Enhanced Salmonella Colonization. PLoS ONE 7(11): e49646.
- Guo, J., Du, L.-X., Ma, Y. –H., Guan, W.-J., Li, H. –B., Zhao, Q. –J., Li, X., Rao, S.-Q., (2005). A novel maternal lineage revealed in sheep (*Ovisaries*). International Society for Animal Genetics, Animal genetics. 36: 331-336.
- 10. Mona S. Z., Nagwa S. A., Shalaby, S. I.andEiman M. Z. (2010). Diarrhoea in Neonatal baraki kids-goats. Life Science Journal, Vol 7, No 3. http://www.sciencepub.net.
- 11. Radostits, O.M., C.C. Gay, K.W. Hinchcliff and P.D. Constable. (2007). In: Veterinary Medicine, A textbook of the diseases of cattle, horses, sheep, pigs, and goats. Saunders, Elsevier Ltd. 10 Edi. pp, 314, 316, 321, 898, 1541.
- 12. Rosallie, T; Matthew, K. and Carol, K. (1985). Effect of age and fever and acute-phase response of rat to endotoxin and salmonella typhimurium. Inf. And Imm. Vol. 47: 106-111.
- 13. Santos R. L. Zhang S. Tsolis R. M. Baumler A. J. Adams L. G. (2002). Morphological and molecular characterization of salmonella typhimurium infection in neonatal calves. Vet. Patho. 39: 200-1510.
- 14. **Tauxe, R.** (1999).*Salmonella enteritidis* and *Salmonella typhimurium* DT104. Successful subtypes in the modern world. In: Emerging infection. Washington. Asm. Press. P: 37-52
- 15. OhI, M. E. and Miller, S.I. (2001). *Salmonella*: a mode for bacterial pathogenesis Annul. Rev. Med., 52: 259-274.
- Ansaruzzaman, M.; Albert, J.; Nahar, S.; Byun, R.; Katouli, M.; Kuhn, and Mollby, R. (2000). Clonal groups of enteropathogenic isolated in case control studies of diarrhea in Bangladesh .J. Med. Microbial., 49:177-185
- 17. Coles, E.H. (1986). Veterinary Clinical Pathology. 4th edition W.B Sounders Co. USA. 486
- 18. **Balk, R. A. (2002).**Endotoxemia in critically ill patients. Why a reliable test could be beneficial. Crit. Care,6: 289-290.
- Kramer, B.W.; Moss, T.J.; willet, K.E.; Newnham, J.P.; Sly, P.D.;Kallapure, S.G.; Ikegami, M. and Job, A. H. (2001). Dose and time response after intra amniotic endotoxin in preterm lambs. Am. J. Respir. Crit. Car Med, 164 (6): 289-988.
- 20. Thomas D Coates. (2016).Infectious causes of neutropeinia .Literature review. University of southern California school of medicine .UP todate website April 2016.
- Zhao, C., B.J. Ge, R. De Villena, E. Sudler, S. Yeh, D.G. Zhao, D. White, Wagner and J. Meng, (2001). Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella serovars*in retail chicken, turkey, pork, and beef from the greater Washington, D.C., area. Appl. Environ. Microbiol. 67: 5431-5436.