# Study the Immunohistochemical Changes in White Mice Following Experimental Infection with Shigatoxin Producing Escherichia Coli (Stec) 0157: H7

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**Abstract**
This study was designed to find immunohistochemical changes in mice organs following experimental infection with shiga toxin Escherichia coli 0157:H7.For this reason two groups of white mice, one month old, Kept for 2 weeks for acclimation The first group infected orally with 2ml ( 2.5 x$10^5$) infective dose ( LD50 =2.5x $10^8$ ) of STEC 0157: H7 the 2nd group drenched with 1ml of phosphate buffered saline as a control All groups were sacrificed at 7th,14th,21th and 28th days and pieces of tissue from different organs were taken and stained immunochemically the result showed CD8+ and CD4+ T cells markers were present in all organs with different periods of infection, the positive CD8+ and CD4+ T cells markers appeared as brown dots on the surface of cells in examined immune staining tissues comparable not brown dots i.e Negative CD8+ and CD4+ T cells markers in control group.
Keywords: STEC 0157:H7, Immuno-histochemical changes.

## 1. Introduction

Shigatoxin producing Escherichia coli ( STEC) infection has been described in a wide range of both domestic, wild animals and human, The animals ( cattle ) being recognized as the major reservoir for human infections (1),(2),(3).Human infection with STEC cause serious diseases (4) such bloody diarrhea hemolytis uremic syndrome, hemorrhagic colitis, Thrombotic thrombocytopenic purpura, fever, vomiting and possible death (5),(6),(7),(8),(9).

The STEC were classified depending on the importance of serotype 0157:H7 in human diseases into two Major categories,STEC 0157 and non-0157 STEC (10),(11). Most body response against STE involve two arms of immunity humoral and cellular immunity and these immune responses both T cell CD4+,CD8+ proliferation primary that during an infection both these cells regulate the selective production of cystokines and chemokines that attract, activate and retain specific subsets of the effector immune cells.CD8+ provide protection against number of viral, bacterial pathogens and even against Tumor cells (12) when rechallenged with same infectious pathogen, the antigen specific memory T cells can respond swiftly and regulate the effector function (13) although the events required for the initiation of a primary CD8+ T cells response have been extensively studied the cellular and molecular mechanism that initiate and prepetuate an effective memory CD8+ T cells recall response and help are less defined which is the aim of this study:

To demonstrate the level appearance of both CD4+ and CD8+ response against STEC 0157 infection in white mice in the different organs.

## 2. Materials and Methods

Two groups of white mice ( 30 each group, 4weeks old, were taken and drinched with 1ml of streptomycin (6mg/ml) and after one day drinched with 1 ml of 2.5x$10^5$ ( CFU /ml) of shiga toxin producing Escherichia coli 0157:H7 an infective dose (14) the other group were drenched with Iml of phosphate buttered saline.After 7 days,14days,21 days and 28 days the animals were sacrificed and pieces of different organs were taken for preparation tissue section for histopathology and parafinized tissue section were taken for immunohistochemistry (15).to demonstrate level appearance of CD4+ and CD8 +T cells in different tissue sections.

### Immunohistochemical procedure:

Preparation of immunostain formalin – fixed, paraffin – embedded tissue sections:

1. De paraffinize in 2 changes of xylene,5 min each.
2. Transfer slides to 100% alcohol, for 2 changes 3 min each, and then transfer once through 95%,70% and 50% alcohol, respectively for 3 min each.
3. Block endogenous peroxidase activity by incubating sections in 3% H2O2 solution in methanol at room temp, for 10 min to block endogenous peroxidase activity.
4. Rinse in 300 ml of PBS for 2 changes,5 min each
5. (Optional) perform antigen retrieval to unmask the antigenic epitope.The most commonly used antigen retrieval is a citrate buffer method. Arrange the slides in a staining container, Pour 300 ml of 10 m M citrate buffer.Ph 6.0 into the staining container and incubate it at 95-100$^0$ C for 10 minutes.Remove the staining container to room temp, and allow the slides to cool for 20 minutes.
6. Rinse slides in 300 ml PBS for 2 changes, 5 min each.

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7. (Optional) add 100 μL blocking buffer (e.g10% mouse serum in PBS) onto the sections of the slides and incubate in a humidified chamber at room temp for 1 hr.
8. Drain off the blocking buffer from the slides.
9. Apply 100 μL appropriately diluted primary antibody (mouse anti-rat CD8 antiboy) (DAKO/Denmark) to the sections on the slides and incubate in a humidified chamber at room temp for 1hr.
10. Wash the slides in 300 ml PBS for 2 changes 5 min each.
11. Apply 100 μL appropriately diluted biotinylated secondary antibody (Envision DAKO/Denmark) (using the antibody dilution buffer) to the sections on the slides and incubate in a humidified chamber at room temp for 30 min.
12. Wash slides in 300 ml PBS for 2 changes 5 min each
13. Apply 100 μL substrate solution to the sections on the slides to reveal the color of antibody staining.Allow the color development for 5 min until the desired color intensity is reached.
14. Wash slides in 300 ml PBS for 3 changes 2 min each.
15. Counterstain slides by immersing slide in hematoxylin for 1-2 min
16. Rinse the slides in running tap water for 15 min.
17. Dehydrate the tissue through 4 changes of alcohol (95%,95%,100% and 100%).5 min each.
18. Clear tissue slides in 3 changes of xylene, 5 min each.
19. Observe the color of the antibody staining in the tissue sections under light microscope on oil lens and detection of CD8+, CD4+ markers antigen in the histological sections which appear as small dots on the surface of lymphocytes of spleen.

## 3. Results and Discussion

The results of this study revealed that CD4+ and CD8+ T Cells markers were detected in the different tissue sections in the infected group of mice with STEC 0157: H7 whereas few or mild appearance of CD8+, CD4+ T cells markers in the different tissue section in control group.

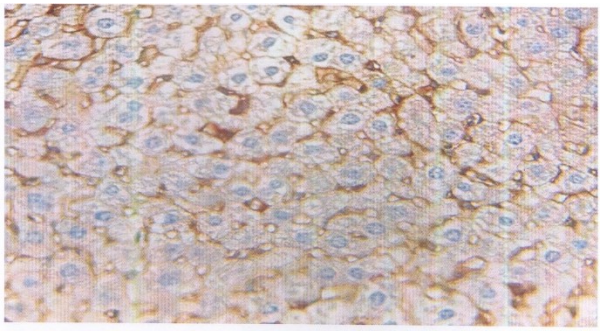

*Figure -1 Microscopic appearance of liver of the control mouse parenchyma negative for CD4, 400 X*

The CD8+ and CD4+ T cells markers appeared as brown dots on cells of tissue section of the different organs.

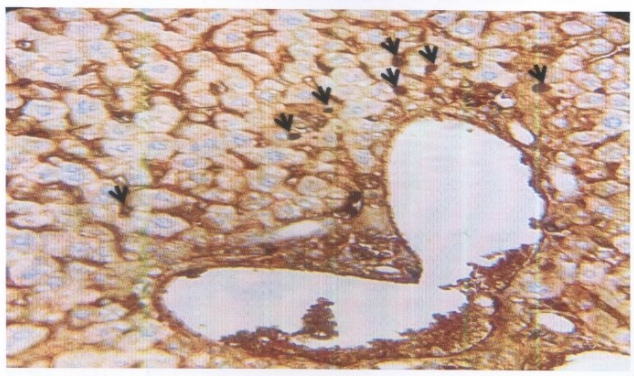

*Fig-2: Microscopic appearance of liver of the infected mouse with mild centrilobar inflammation positive for CD8 (Black arrows), 400X*

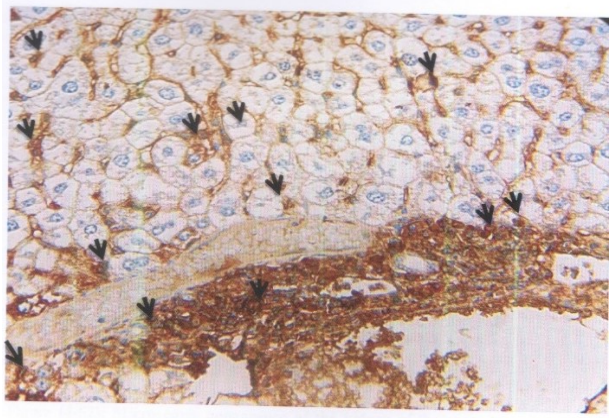

*Figure-3: Microscopic appearance of liver of the infected mouse with moderate inflammation positive for CD4 (Black arrows).400X.*

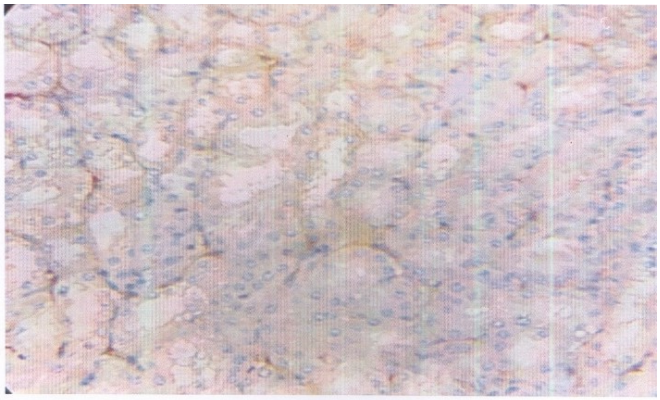

*Figure -4: Microscopic appearance of the kidney of the control mouse negative for CD4, 400X*

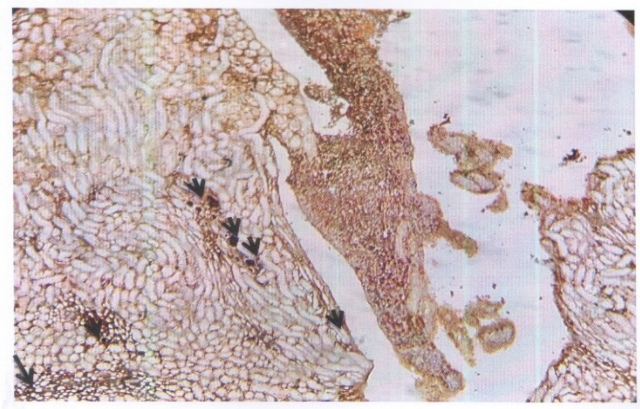

*Figure -5: Microscopic appearance of kidney of the infected mouse with severe inflammation positive for CD4 (Black arrows), 400X*

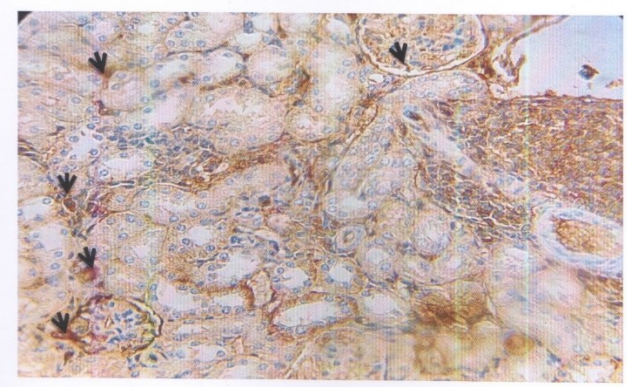

*Figure (6): Microscopic appearance of kidney of the infected mouse with moderate inflammation positive for CD8 (Blank arrows),100X.*

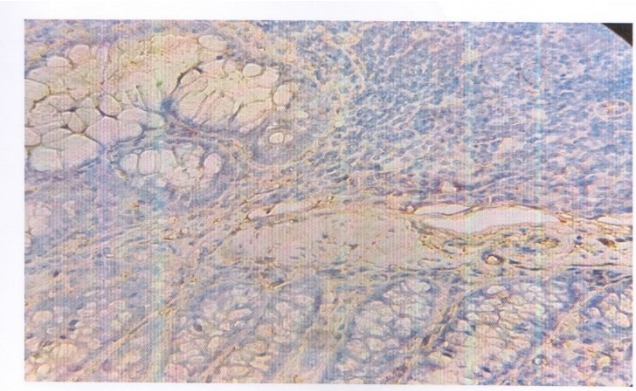

*Figure-7: Microscopic appearance of large bowel of the infected mouse with mild inflammation negative for CD4, 400X*

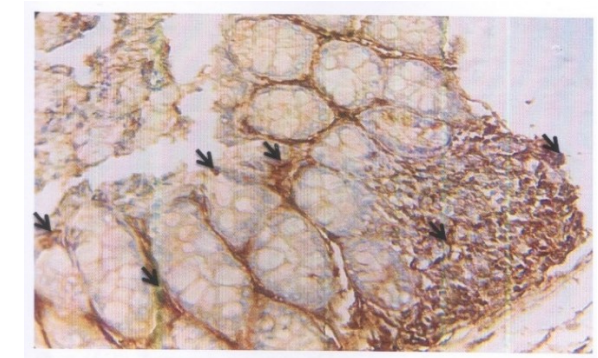

*Figure-8: Microscopic appearance of large bowel of the infected mouse with moderate inflammation positive for CD8 (Blank arrows).100 X.*

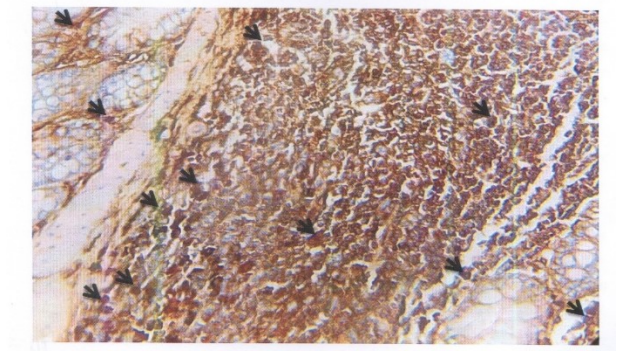

*Figure -9: Microscopic appearance of large bowel of the infected mouse with severe inflammation positive for CD4 (Black arrow).400X*

During the infection of white mice with shigatoxin producing E-coli (STEC) both arms of immunity were appeared humoral immunity with antibody with cytokines production and immunohistochemical cells proliferation (CD8+ T cells) with initiation of helper CD4+ T cells. both histochemical Cells appear following precessing antigen at site of presence of microorganism (STEC) (16),(17) by class1 Major histocompatibility ( MHC-1) on surface of processing cells macrophages, dendritic cells both phagocytosed antigen to induce immune response in which T helper CD4+ T cells cooperate to stimulate or recruit CD8+ at the site of infection with STEC in the different organs, which more evident In this study in the different organs similar observation were noted by (17),(18),(19) and (17),(18)

The dependence of antimicrobial CD8+ T cells recall response on CD4+ T cells has been controversial. In some cases CD4 T cells assist the proliferation CD8 T cell recall response (12),(17); studies have also suggested that CD8 memory T cells development is dependent on the presence of CD4+ T cells which was more evident in this study, both sets of CD8+, CD4+ T cells were demonstrated in all tissues of infected group of mice, also after activation of lymphoid organs with any antigen or any microbial agents, most of activated CD8+ and CD4+ T cells will migrate to other target organs when infection occurred (12)

## References

Caproili,A; Morabito,S ; Brugre.,H.and Oswald,E ( 2005) Enterohemorrhagic Escherichia coli: emerging issue on virulence and modes of transmission Vet.Res. 36:289-311

Beutin,I ; Krause, G ; Zimmermann,S ; Kaulfuss, S. and Gleir,K. (2004) characterization of shigatoxin producing Escherichia coli strains isolated from human patients in Germany over a 3 year period.J. Clin. Microbiol.42:1099-1108.

Wang,J;Niu,Y; Chen,J; Anany,H;Ackermann,H.; Johnson,R;ATeba, C.;Stanford,K and Mc Allister,T (2015) Feces of fedlot cattle contain a diversity of bacteriophages that lyse non-0157 shiga toxin producing Escherichia coli.can.J Microbiol.61:467-475.

Beutin,L. (2006) Comparative evaluation of the Ridascreen Vero-toxin enzyme immunoassay for detection of shiga toxin producing Escherichia coli (STEC) from food and other sources J.Appl. Microb.102: 630-639.

Stromberg,Z.R.(2015).Detection methods,and intestinal adherence of non -0157 shiga toxin producing Escherichia coli.Disserations in Vet.and Bio.Sci.Lincon, Nebraska.

Brook,J.; Sowers,E; wells,J; Greene,K,Griffin,P.M ; Hekstra, R.M and Strockbine,N,A (2005) Non-0157 shiga toxin producing Escherichia coli infection in the united states 1983-2002.J.Clin Infect.Dis.92:1422-1429.

Mathusa,E.C; Chen,Y-H; Enache, E and Hontz,L(2010) Non-0157 shiga toxin producing Escherichia coli in foods J.of food protection 73(9) 1721-1736.

Etcheverria,A,I and padola, N. (2013) shiga toxin producing Escherichia coli factors involved in virulence and Cattle colonization.Virulence 4: 366-372.

Croxen,M.A ; Law,R.J.; Scholz,R.Keeney,K.M, Slodarska,M. and Finlay B.B., (2013) Recent advances in understanding enteric pathogenic Escherichia coli. Clin. Microbiol.Rev.26:622-880.

Karmali,M.A; Petric,M; Lim,C ; Fleming,P.C,Arbus,G.S.and Lior,H. (2004) The association between idiopathic hemolytic uremic syndrome and infection by verotoxin producing Escherichia coli J.Infect.Dis 189(3):556-563.

Bettelheim,K.A (2003) non 0157 verotoxin producing Escherichia coli: a problem, paradox and paradigm.Exp.Biol. and Medicine 228(4) 333-344.

Li,Y; Frey,F ; Mackenzie,A.M. and Finlay B.B.(2002) human response to Escherichia coli 0157:H7 infection:antibodies to secreted virulence factor Infect. Immun.68 (9): 5090-5095.

Erickson,M.C and Doyle,M.P (2007) food as a vehicle for transmission of shiga toxin producing Escherichia coli J.food Prot.70: 2426-2449.

Yosif,A.A; and AL Taii,D.H (2014). isolation and characterization of E-coli 0157:H7 from human and animals MRVSA 3(2):11-18.

Leong,ASY; Cooper,K; and Leong,FJW-M (2003).Manual of diagnostic antibodies for immunochemistry 2nd.ed.London Greenwich,Medical Media.LTD.73-74.

Abbas,A.K; Lichtman,A.H. and Pillia,S (2010) cellular and molecular Immunity.updated 6th-ed. Saunders Elseiver Inc.pp:116-117.

Wang, J.H. and ElReinherz (2002) Structural basis of T cell recognition of peptides bound to MHC molecules. Molecular Immunology 38: 1039-1049.

William, M. A; Bevan,M.J.( 2007) effector and memory CTL differentiation.Annual Review of immunology 25: 171-192.

Wong, P; and Pamer,E.G,(2007) CD8+ T cell response to infectious pathogens. Annual Review of Immunology 21:29-70.