



Kafrelsheikh University  
Faculty of Veterinary Medicine  
Clinical Pathology Department

## **Clinicopathological and molecular studies on some microbial enteritis in lambs**

*A Thesis presented by*

**Nahawand Abd Elgwad Elsheikh**  
(B. V. Sc., Kafrelsheikh University-2008)

*Under Supervision of*

**Prof./ Dr. Abd Allah A. Mokhbatly**  
*Prof. and Head of Clinical Pathology Dept.  
Faculty of Veterinary Medicine  
KafrelSheikh University*

**Dr./ Doaa Hosny Abd Elbary**  
*Ass.Prof. of Clinical Pathology  
Faculty of Veterinary Medicine  
KafrelSheikh University*

**Dr./ Adel Mohamed Elgaml**  
*Senior Researcher of  
Bacteriology Animal Health  
Reasearch Institute, Kafrelsheikh*

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## List of Abbreviations

|               |  |
|---------------|--|
| <b>ALP</b>    | Alkaline Phosphatase                       |
| <b>ALT</b>    | Alanine aminotranferase                    |
| <b>AST</b>    | Aspartate aminotransferase                 |
| <b>A/E</b>    | attaching and effacing                     |
| <b>AAFs</b>   | aggregative adherence factors              |
| <b>4AAP</b>   | 4 Amino antipyrine                         |
| <b>BCG</b>    | Bromocresol green                          |
| <b>bfp</b>    | bundle forming pilus                       |
| <b>CAT</b>    | Catalase                                   |
| <b>DCs</b>    | Dendritic cells                            |
| <b>DNA</b>    | Deoxyribo nucleic acid                     |
| <b>eaeA</b>   | Intimin gene                               |
| <b>EAEC</b>   | Enteraggregative <i>Escherichia coli</i>   |
| <b>EDTA</b>   | Ethylene diamine tetra-acetic acid         |
| <b>EHEC</b>   | Enterohaemorrhagic <i>Escherichia coli</i> |
| <b>EIEC</b>   | Enteroinvasive <i>Escherichia coli</i>     |
| <b>eNOS</b>   | Endothelial Nitric oxide synthase          |
| <b>EPEC</b>   | Enteropathogenic <i>Escherichia coli</i>   |
| <b>ETEC</b>   | Enterotoxigenic <i>Escherichia coli</i>    |
| <b>GI</b>     | Gastrointestinal                           |
| <b>GOD</b>    | Glucose oxidase                            |
| <b>GSH-Px</b> | Glutathione peroxidas                      |
| <b>GSH-R</b>  | Reduced glutathione                        |
| <b>Hb</b>     | Hemoglobin                                 |
| <b>HGT</b>    | Horizontal gene transfer                   |
| <b>hlaA</b>   | Hyper-invasive locus gene                  |
| <b>IgG</b>    | Immunoglobulin G                           |
| <b>iNOS</b>   | Inducible Nitric oxide synthase            |



|                          |   |
|--------------------------|---|
| <b>LT</b>                | Heat labile                                 |
| <b>MDA</b>               | Malondialdehyde                             |
| <b>NANC</b>              | Non-adrenergic Non-cholinergic Transmission |
| <b>NO</b>                | Nitric oxide                                |
| <b>NO<sub>2</sub></b>    | Nitrite                                     |
| <b>NO<sub>3</sub></b>    | Nitrate                                     |
| <b>NOS</b>               | Nitric oxide synthase                       |
| <b>nNOS</b>              | Neuronal Nitric oxide synthase              |
| <b>O-H antigens</b>      | Somatic-Flagellar Antigens                  |
| <b>OF</b>                | Oxidation fermentation                      |
| <b>PCR</b>               | Polymerase chain reaction                   |
| <b>PCV</b>               | Packed cell volume                          |
| <b>POD</b>               | Peroxidase                                  |
| <b>RBCs</b>              | Red blood cells                             |
| <b>SOD</b>               | Super Oxide Dismutase                       |
| <b><i>S.enterica</i></b> | <i>Salmonella enterica</i>                  |
| <b>STEC</b>              | Shigatoxigenic <i>E. coli</i>               |
| <b>ST</b>                | Heat stable                                 |
| <b>stx1, stx2</b>        | Shiga toxin 1,2                             |
| <b>stn</b>               | Enterotoxin gene.                           |
| <b>TBARs</b>             | Thio barbituric acid reactive substance     |
| <b>TBE</b>               | Tris boric acid EDTA                        |
| <b>TLC</b>               | Total leucocytic count                      |
| <b>TSI</b>               | Triple sugar iron                           |
| <b>μl</b>                | Microlitter                                 |
| <b>VTEC</b>              | Verocytotoxin-producing <i>E. coli</i>      |
| <b>WBCs</b>              | White blood cells                           |
| <b>XLD agar</b>          | Xylose Lysine Deoxycholate agar             |

## **6- SUMMARY**

Bacterial enteritis is a serious problem facing the intentional intensified livestock production and practical and economic efforts are much required to minimize the disease morbidity and mortalities resulting mainly from the sever alteration in the hemato-biochemical parameters and oxidative-antioxidative balance in affected lambs.

Alterations in the hemato-biochemical parameters in the case of bacterial enteritis among neonatal sheep is a serious substantial cause of lambs' morbidity and mortality around the world.

This study was intended to investigate the prevalence of major bacterial causes of bacterial enteritis among lambs in Egypt; *Escherichia coli* (*E. coli*) and *Salmonellae* spp. and to determine the associated changes in hemato-biochemical and oxidative parameters.

A total number of 70 lambs (50 diarrheic and 20 apparently healthy (control lambs) at Kafrelsheikh Governorate, Egypt were subjected to clinical examination and blood and serum samples were collected to describe the hemato-biochemical and oxidative parameters. Rectal swaps were collected from diarrheic lambs to isolate the causative agents of enteritis and detection of the virulence genes of Shiga toxin -producing *E. coli* (STEC) and *Salmonellae* Spp.

Results showed that the diarrheic lambs had a significant increase in body temp, respiratory rate and pulse rate. There was a significant increase in hemogram parameters in diseased lambs than control ones while for total leukocytic count (TLC) and lymphocytes which were significantly reduced. Moreover, significant increase in total proteins and albumin and significant decrease in glucose concentrations was detected in diseased lambs compared to normal control lambs. In diseased lambs,

oxidative stress markers malonaldehyde (MDA) and nitric oxide (NO) levels were significantly increase, while, antioxidant biomarkers superoxide dismutase (SOD) activities and reduced glutathione (GSH-R) levels were significantly reduced.

In addition, there were significant elevation in liver enzymes alanine amino transferase, aspartate amino transferase and alkaline phosphatase (ALT, AST and ALP) as well as kidney functions (urea and creatinine) levels.

Bacteriological examination of fecal samples revealed that the most common pathogens isolates from the examined lambs were *E. coli* and *Salmonella* spp.; from 32% and 16% of diseased lambs, respectively. Serotyping and biochemical tests of examined samples identified 16 bacterial isolates of *E. coli* belonged to 10 different serotypes; O6, O8, O26:H11, O75, O84:H21, O103:H2, O114:H4, O121:H7, O128:H2 and O163:H2.

Molecular characterization of *E. coli* isolates revealed that all of isolates are STEC as they harbor either *stx1* or *stx 2* genes or both of them. One of them carry intimin gene *eaeA* and classified as EHEC; O26:H11. Serotyping and biochemical tests of examined samples identified 9 bacterial isolates of *Salmonella*, which belonging to 6 different serotypes.

Molecular analysis showed a number of *Salmonella* isolates that carry (*stn*) genes belonged to serogroups; *S. Enteritidis* (mainly), *S. Heidelberg*, *S. Tsevie*, *S. Typhimurium*. On the contrary, the production of (*hilA*) gene was shown among the recovered serogroups: *S. Enteritidis* (mainly), *S. Heidelberg*, *S. Essen*, *S. Typhimurium* and *S. Infantis*.