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

Submitted to
Master Degree in Veterinary Medical Sciences
(Clinical Pathology)
Faculty of Veterinary Medicine
Kafrelsheikh University- Egypt

2020

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List of Abbreviations

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

LT	Heat labile
MDA	Malondialdhyde
NANC	Non-adrenergic Non-cholinergic Transmission
NO	Nitric oxide
NO2	Nitrite
NO3	Nitrate
NOS	Nitric oxide synthase
nNOS	Neuronal Nitric oxide synthase
O-H antigens	Somatic-Flagellar Antigens
OF	Oxidation fermentation
PCR	Polymerase chain reaction
PCV	Packed cell volume
POD	Peroxidase
RBCs	Red blood cells
SOD	Super Oxide Dismutase
S.enterica	*Salmonella enterica*
STEC	Shigatoxigenic E. coli
ST	Heat stable
stx1, stx2	Shiga toxin 1,2
stn	Enterotoxin gene.
TBARs	Thio barbituric acid reactive substance
TBE	Tris boric acid EDTA
TLC	Total leucocytic count
TSI	Triple sugar iron
µl	Microlitter
VTEC	Verocytotoxin-producing *E. coli*
WBCs	White blood cells
XLD agar	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 malonaldiahyde (MDA) and nitric oxide (NO) levels were significantly increase, while, antioxidant biomarkers super oxide 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*.