

CONTENTS

Item	Page
INTRODUCTION.....	1
REVIEW OF LITERATURE.....	4
1. History of Johne’s disease	4
2. The economic impact of paratuberculosis	4
3. Epidemiology of Paratuberculosis	6
3.1. Prevalence of paratuberculosis	10
4. Diagnosis of paratuberculosis.....	11
4.1. Clinical signs and presentation.....	11
4.2 Acid fast staining of clinical samples.....	13
4.3. Phenotypic characterization and identification of MAP.....	14
4.4. Detection of MAP specific antibodies using ELISA.....	19
4.5. Genotyping identification and molecular characterization of MAP.....	23
MATERIAL AND METHODS.....	28
1. Material.....	28
2. Methods.....	36
EXPERIMENTS AND RESULTS.....	43
DISCUSSION.....	67
ENGLISH SUMMARY.....	72
REFERENCES.....	74
ARABIC SUMMARY.....	99

LIST OF ABBREVIATIONS

AGID	Agar gel immunodiffusion test
Bp	Base pair
CD	Crohn's disease
CFT	Complement fixation test
CFU	Colony forming unit
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent assay
HEYM	Herrold's egg yolk medium
HPC	Hexadecylpyridinium chloride
HSe	Herd sensitivity
HSp	Herd specificity
IgG	Immunoglobulin G
IS900	Insertion sequence 900
JD	Johne's disease
LJ	Lowenstein –Jensen medium
MAP	<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i>
MAC	<i>Mycobacterium avium</i> complex
NAHMS	National Animal Health Monitoring System
NVSL	National Veterinary Services Laboratories
OIE	Office Internationale des Epizooties
PCR	Polymerase Chain Reaction
PFGE	Pulsed –field gel electrophoresis
PTB	Paratuberculosis

QRT-PCR	Quantitative Real time Polymerase Chain Reaction
RFLP	Restriction fragment length polymorphism
TB	Tuberculosis
TRT-PCR	Triplex Real time Polymerase Chain Reaction
USDA	U.S.Department of Agriculture
ZN	Zeihl-Neelsen stain

LIST OF TABLES

Item	Page
Table (1) Type and No. of animals and sample.	29
Table (2) Primers sequence used in PCR	33
Table (3) Conjugate preparation	39
Table (4) PCR cycling protocol for IS900- gene of MAP using a conventional PCR	42
Table (5) The numbers of pooled fecal samples from both clinically diseased and apparently healthy buffaloes	43
Table (6) Results of bacteriological examination of pooled fecal samples	45
Table (7) Results of bacteriological examination of the pooled fecal samples of the clinically diseased buffaloes	46
Table (8) Results of bacteriological examination of pooled fecal samples from apparently healthy buffaloes	47
Table (9) Results of Ziehl -Neelsen staining of the pooled fecal samples.	50
Table (10) Results of ZN-staining of the pooled fecal samples of clinically diseased buffaloes	51
Table (11) Results of ZN-staining of the pooled fecal samples of the apparently healthy buffaloes	52
Table (12) Results of PCR of the pooled fecal samples	57
Table (13) Results of PCR of the pooled fecal samples of the clinically diseased buffaloes	58
Table (14) Results of PCR of the pooled fecal samples of the apparently healthy buffaloes	59
Table (15) Results of indirect ELISA of the total collected sera samples of both clinically diseased and apparently healthy buffaloes	62
Table (16) Results of indirect ELISA of the tested sera samples of clinically diseased buffaloes	64
Table (17) Results of indirect ELISA of the tested sera samples of apparently healthy buffaloes	65
Table (18) Comparison between results of bacteriological culture , ZN staining and PCR of the pooled fecal samples	66

LIST OF FIGURES

Item	Page
Fig. (1): Results of culture examination of the pooled fecal samples	45
Fig. (2): Results of culture examination of the pooled fecal samples of clinically diseased buffaloes.	46
Fig. (3): Results of culture examination of the pooled fecal samples of the apparently healthy buffaloes.	48
Fig. (4): Results of Ziehl -Neelsen staining of pooled fecal samples.	51
Fig.(5): Results of ZN-staining of the pooled fecal samples of the clinically diseased buffaloes.	52
Fig.(6): Results of ZN-staining of the pooled fecal samples of the apparently healthy buffaloes.	53
Fig. (7): The ZN – staining technique sensitivity, specificity and accuracy regarding the clinically diseased animals	54
Fig. (8): The ZN sensitivity, specificity and accuracy regarding the apparently healthy animals.	54
Fig. (9): The overall ZN sensitivity, specificity and accuracy.	54
Fig. (10): Results of PCR of the pooled fecal samples.	57
Fig. (11): Results of PCR of the pooled fecal samples of the clinically diseased buffaloes.	58
Fig. (12): Results of PCR of the pooled fecal samples of the apparently healthy buffaloes.	59
Fig. (13): Sensitivity, specificity and accuracy of PCR regarding the clinically diseased buffaloes.	60
Fig: (14): Sensitivity, specificity and accuracy of PCR regarding the apparently healthy animals.	60
Fig (15): The overall sensitivity, specificity and accuracy of PCR.	61
Fig. (16): Results of indirect ELISA of the total collected sera samples of both clinically diseased and apparently healthy buffaloes.	63
Fig. (17): Results of indirect ELISA of the tested sera samples of clinically diseased buffaloes.	64
Fig. (18): Results of indirect ELISA of the tested sera samples of apparently healthy buffaloes.	65
Fig.(19): Comparison between results of bacteriological culture, ZN staining and PCR of the pooled fecal samples	66

LIST OF PHOTOS

Item	Page
Photo (1) Buffalo suffering from chronic diarrhea and emaciation.	28
Photo (2): Typical colonies of MAP on Herrold's Media; very small, convex (hemispherical), soft, non mucoid and initially colourless and translucent.	44
Photo (3): presence of Acid fast resistant bacilli in a buffalo fecal smear subjected to Ziehl-Neelsen staining.	50
Photo (4.1): Agarose gel of IS900 PCR amplicons of some fecal samples.	56
Photo (4.2): Agarose gel of IS900 PCR amplicons of some fecal samples.	56

Cairo University
Faculty of Veterinary Medicine
Department of Medicine and Infectious Diseases

Name:Shohanda Moustafa Kamel Bayoumi

Nationality: Egyptian

Date of Birth: 1/8/1989

Place of Birth: Cairo, Egypt

Specialization: Infectious diseases

Degree: M.V.Sc

Title of M.V.Sc. thesis:

Role of *Mycobacterium avium* subspecies *paratuberculosis* in persistent diarrhoea in Egyptian buffaloes

Supervisors:

Prof.Dr.Samia Abd El Hamid Ahmed

Professor of Infectious Diseases, Faculty of Veterinary Medicine, Cairo University.

Dr.soliman Mohammed Soliman

Lecturer of Infectious Diseases, Faculty of Veterinary Medicine, Cairo University.

Dr.Attia Abd Allah El Gedawy

Senior Researcher of Bacteriology, Animal Health Research Institute, Dokki.

Abstract

Paratuberculosis or John's disease (JD) is a chronic and incurable granulomatous enteric disease affecting cattle, buffaloes, sheep, goats and other ruminants caused by *M.avium* subspecies *paratuberculosis* (MAP) . In this study, A total of 300 buffaloes (240 clinically diseased animals and 60 apparently healthy animals) were examined for the presence of clinical signs of John's disease including incurable chronic diarrhoea, intermittent firstly then intense and continuous which is not responding to treatment, emaciation and progressive weakness . Fecal and serum samples (each of 300) were collected from the examined buffaloes housed in three Egyptian Governorates (Al-Sharkia, Al-Kalyoubia and Damietta). Fecal samples were collected then examined according to the pooling procedure and decontaminated by Hexa decylpyridinium chloride solution (HPC 0.9%) prior to culturing on Herrold's Egg Yolk Medium (HEYM). MAP was isolated from 34 of the 60 pooled fecal samples tested (57%). Fecal smears were examined using Ziehl – Neelsen stain (ZN) for the presence of acid fast bacilli revealing 29 fecal smears (48%) of 60 fecal smears were positive. ELISA was conducted on serum samples to detect antibodies against MAP, 212 (71%) of serum samples were positive for antibodies against MAP. Molecular confirmation by PCR IS900 assay was carried out using specific primers directly on fecal sample, Out of the 60 pooled fecal samples, 45 pools (75%) were positive. This study aimed to throw a light on paratuberculosis in Egyptian buffaloes as there is lack of data about this disease in Egypt.

Keywords: Paratuberculosis - John's disease- buffaloes-Persistent diarrhoea.