Cairo University

Faculty of Veterinary Medicine

Department of Microbiology

Bacteriological and Molecular Studies on *Streptococcus* Species in Horses.

A Thesis Presented

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(MSc, 2012 Cairo University)

For the degree of

Ph.D. in Veterinary Science

(Bacteriology, Immunology and Mycology)

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2017

ABSTRACT

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In the present study 150 swabs "100 nasal swabs and 50 pus swabs" were collected from horses suffered clinically from strangles symptoms for examination of *Streptococcus* spp**.** The microbiological and biochemical results revealed that there were 2 *S.equi* isolates" all from pus swabs and 2 *S.zooepidemicus* isolates" one from nasal swabs and one from pus swabs " , these result confirmed serologically by using specific antisera and also molecularly by using PCR with specific primers for each species. Antibiogram for the recovered *S.equi* and *S.zooepidemicus* isolates showed that they were sensitive for Penicillin " except one isolates of *S.zooepidemicus* "and cephalosporins " except one isolates of *S.equi* " ., while they show high resistance for bacitracin , erythromycin , tetracycline and amikacin. Three essential oils (cinnamon, anise and oreganium) were tested for their antimicrobial activity against *S.equi and S.zooepidemicus* . Results were recorded that cinnamon oil completely inhibited the growth of all the tested bacterial isolates (100%) at a concentration of 3% and (50%) at a concentration of 2%, on contrary, 1% concentration had no effect on all the tested isolates , while anise oil did not show any growth inhibitory effect at any concentration used in the present study (1%, 2%, 3%, 4%, 5% and 6%), In other hand oreganium oil exerted a strong growth inhibitory effect on *Streptococcus* isolates at concentration of 1% (100%). while at 0.5% concentration has no effect.

ACKNOWLEDGEMENT

First, My Deepest Prayerful Thanks To ***Allah*** For Giving Me Everything I Need.

No Word Can Express My Deepest Appreciation, Gratitude And Sincere Thanks To **Prof. Dr. Mahmoud Essam Hatem And Prof. Dr. Kahled Farouk El Amry** , Professor Of Microbiology, Faculty Of Veterinary Medicine, Cairo University For Their Valuable Supervision, Ideal Guidance, Constructive Criticism And Continual Encouragement Throughout The Course Of The Study Without Which This Thesis Would Not Have Been Fulfilled In Its Present Form.

I Wish To Express My Thanks To **Dr. Hussien Mohamed Galal** Assistant Professor Of Microbiology, Faculty Of Veterinary Medicine, Cairo University For His Valuable Supervision, Guidance, And Continual Encouragement Throughout The Course Of The Study.

I Wish To Express My Thanks To **Ahmed Orabi Hassan ,** Assistant Lecturer Of Microbiology, Faculty Of Veterinary Medicine, Cairo University For His Valuable Help, Advise And Facilities He Offered During The Practical Work.

I Am Very Gratefully Indebted To All Of The Staff Members Of The Department Of Microbiology, Faculty Of Veterinary Medicine, Cairo University Who Were Always Available When I Needed Advice.

LIST OF CONTENTS

Title	Page
INTRODUCTION	1
REVIEW OF LITERATURE	6
MATERIALS And METHODS	49
RESULTS	74
DISCUSSION	84
Conclusion	93
SUMMARY	94
REFERENCES	97
ARABIC SUMMARY	102

LIST OF TABLES

Number	Table	**Page**
1	Mechanism Of Action Of Herbal Drugs	46
2	Numbers Of Examined Samples For Horses	49
3	Oligonucleotide Primer Sequence	55
4	Antibiotics Used In Sensitivity Test	58
5	Interpretation Of Antibiotic Sensitivity Test According To Clinical And Laboratory Standard Institute	59
6	The Polymerase Chain Reaction Conditions For Different Primers	66
7	The Polymerase Chain Reaction Mixture	67
8	Result Of Antimicrobial Sensitivity For Recovered *Streptococcus Spp*	80
9	Detection Of Antibacterial Effect Of Cinnamon On *Streptococcus* Isolates	82
10	Detection Of Antibacterial Effect Of Oreganium Oil On *Streptococcus* Isolates	83

LIST OF FIGURES

Number	Figure	Page
1	Electron Microscope Photo Of Streptococcus In Chains - Streptococcus Equi Subsp. On Horse Blood Agar - Streptococcus Equi Subsp. Zooepidemicus On Horse Blood Agar	1
2	*Enlarged Submandibular Lymph Nodes In Horses*	2
3	Opened Submandibular Ln Foals	2
4	Strep-Check Kit	54
5	Showed Gram-Positive Cocci Of The Suspected *Streptococcus* Isolates	74
6	Showed Small Size Beta Hemolytic Colonies Which Were Suspected As Indicator For The Presence Of *Streptococcus* Spp	75
7	Showed Catalase -Ve For All Suspected *Streptococcus* Isolates	76
8	Latex Agglutination Test	76
9	Agarose Gel Electrophoresis Showing Positive Amplification Of Product 480 Bp Fragment Of 16srrna Gene Of *Streptococcus* Spp. Performed With Specific Primer	77
10	Antimicrobial Sensitivity Test For Recovered *Streptococcus* Isolates On Muller Hinton Agar With 7% Horse Blood	79
11	Summary Of Streptococcus Equi And Zooepidemicus Functional Genes	89

List Of Abbreviations

Abbreviations	Name
Bhi	Brain Heart Infusion
Bsa	Bovine Serum Albumin
Ddh2o	Double Distilled Water
Dna	Deoxyribonucleic Acid
Dntps	Deoxynucleotide Triphosphates
Elisa	Enzyme-Linked Immumosorbent Assay
G	Gram
Hr	Hour (S)
Igg	Immunoglobulin G
Il	Interleukin
Kda	Kilo Dalton
L	Liter (S)
M	Moles Per Liter
Mhc	Major Histocompatibility Complex
Od	Optical Density
Pbs	Phosphate Buffered Saline
Pcr	Polymerase Chain Reactions
Rpm	Revolutions Per Minute
S. Equi	*Streptococcus Equi* Subsp. *Equi*
S. Pyogenes	*Streptococcus Pyogenes*
S. Zooepidemicus	*Streptococcus Equi* Subsp. *Zooepidemicus*

1-Introduction

Streptococcus Equi Subspecies *Equi* (*S. Equi*) Is The Causative Agent Of Equine Strangles, Characterized By Abscessation Of The Lymph Nodes Of The Head And Neck. Rupture Of Abscesses Formed In Retropharyngeal Lymph Nodes Into The Guttural Pouches Leads To A Proportion Of Horses Becoming Persistently Infection Carriers (**Jorm *Et Al* ., 1994**) .These Carriers Transmit The Organism To Naive Horses And Play An Important Role In Disease Spread. *S. Equi* Is Believed To Have Evolved From An Ancestral Strain Of *Streptococcus Equi* Subspecies *Zooepidemicus* (*S. Zooepidemicus*) Which Is Associated With A Wide Variety Of Diseases In Horses And Other Animals Including Humans (**Webb *Et Al* ., 2008)**.

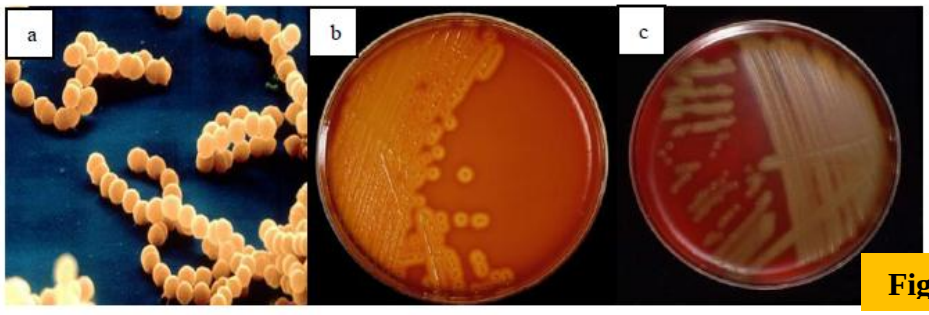

Fig 1

Figure 8. *a) Electron microscopy photo of streptococci in chains; b) Streptococcus equi subsp. equi on horse blood agar; c) Streptococcus equi subsp. zooepidemicus on horse blood agar. (Photo: Bengt Ekberg, National Veterinary Institute, Uppsala, Sweden.)*

Both Of These Organisms Belong To The Same Group Of Streptococci As The Human Pathogen *Streptococcus Pyogenes*.

Previous Work Has Shown That *S. Equi* Produces Four Superantigens (Seeh, Seei, Seel And Seem) , Two Secreted Fibronectin Binding Proteins (SFS And FNE) , A Novel M-Protein (Sem) , An H-Factor-Binding Protein (Se18.9) And A Novel Non-Ribosomal Peptide Synthesis System , But Little Is Known About Other Factors That Influence Differences In The Virulence Of These Closely Related Streptococci. They Was

Determined Complete Genome Sequence Of *S. Equi* Strain 4047 (Se4047) (**Alber *Et Al* ., 2005)**, A Virulent Strain Isolated From A Horse With Strangles In The New Forest, England, In 1990 And *S. Zooepidemicus* Strain H70 (Szh70), Isolated From A Nasal Swab Taken From A Healthy Thoroughbred Racehorse In Newmarket, England, In 2000 (**Artiushin *Et Al* ., 2002)** . Using Comparative Genomic Analysis To Identify Se4047-Specific Loci, And Subsequent Screening Of *S. Equi* And *S. Zooepidemicus* Strains From Around The World, An Evidence Of The Genetic Events That Have Shaped The Evolution Of The *S. Equi* Genome, And Led To Its Emergence As A Host-Restricted Pathogenic . (**Proft *Et Al*., 2003**).

Strangles Is An Important Infectious Disease Affecting Horses. It Is Caused By *Streptococcus Equi* Subspecies *Equi*, A Member Of The Lancefield C Group Streptococci, And Is Characterized By An Acute, Febrile, Suppurative, Lymphadenitis (**Harrington *Et Al* ., 2002)**. Horses Suffering From Strangles Have Mucopurulent Nasal Discharge And Abscesses, Which May Often Burst And Exude. Affected Populations Present High Morbidity Levels And Infection May Cause Chronic Illness Or Even Death. Strangles Is Very Contagious, Especially With

Fig (2): enlarged submandibular L.N in

Fig (3): opened submandibular L.N

Foals, Spreading Easily From Horse To Horse And Often Leading To Large Outbreaks (**Sweeney., 1996)**.It Has Been Recommended That Horses Be Included In Regular Vaccination Programmes But The Efficacy Of Most Available Parenterally Administered Vaccines Has Been Disappointing. These Include The Use Of *S. Equi* M-Like Protein (Sem) Rich Extracts, Which Have Not Made Major Improvements In The Control Of The Disease Because Of Failure To Produce Mucosal Antibodies (**Nally *Et Al* ., 2002)**. In Addition, These Vaccines May Have Undesirable Side Effects Such As Adverse Reactions At The Site Of Injection While Vaccinated Horses May Still Develop Clinical Strangles. Locally Produced Nasopharyngeal Antibodies Play An Important Role In The Immune Response Of Horses To Protein Antigens Of *S. Equi*, Suggesting The Nasal Mucosa As A Promising Immunization Route (**Sheoran *Et Al* ., 2002)**. Effective Immunization Against *S. Equi* May Therefore Depend On The Successful Induction Of A Mucosal Immune Response. Immunizing Via Mucosal Routes To Stimulate The Mucosal Immune System Offers Several Advantages Over Parenteral Vaccination, Such As Improved Efficacy, Ease And Economics Of Preparation And Dosing, And Reduced Side Effects. An Attenuated Live *S. Equi* Nasal Vaccine Has Been On The Market Since 1998 (**Pinnacle IN_, Fort Dodge Laboratories, USA**) But Its Use Has Raised Important Questions About Its Safety, Due To Reactions Including Nasal Discharge, Abscessation Of Lymph Nodes And Other Sites, Allergic Reactions, Systemic Responses And Purpura-Like Signs (**Flock *Et Al* ., 2004)** .

S. Zooepidemicus And Its Host Restricted Clonal Derivative *S. Equi* (**Webb *Et Al.*, 2008**) Share Over 98 % DNA Sequence Homology And Express Many Of The Same Or Similar Proteins. Although Closely Related These Organisms Exhibit Important Differences In Pathogenesis, Host Pathogen Interaction, And Ability To Activate Immune Responses Effective In Clearance. Furthermore, Despite Their High Genetic Homology And Similar Protein Profiles, Infection By *S. Zooepidemicus* Does Not Confer Protection Against *S. Equi* And Vice Versa. *S. Zooepidemicus* Is The Most Frequently Isolated Opportunistic Pathogen Of Horses (**Timoney *Et Al.*, 2004**) And Unlike *S. Equi* Permanently Colonizes The Equine Tonsil And Other Body Surfaces. On The Other Hand, *S. Equi* Is Highly Contagious Causing Purulent Tonsillitis And Pharyngitis With Metastasis To One Or More Lymph Nodes Of The Head And Neck. Failure Of Drainage /Clearance Of The Guttural Pouch May Result In A Chronic Carrier State. In Donkeys, *S. Equi* Infection Is Described As A More Caseous Form Of Lymphadenitis And Respiratory *S. Zooepidemicus* Infection With More Severe Bronchopneumonia Than In Horses. Unlike *S. Zooepidemicus, S. Equi* Does Not Typically Persist In The Tonsils And Has A Very Limited Survival Time Outside Its Equine Host. Horses That Maintain The Organism Within The Guttural Pouch And/Or Cranial Sinuses Are Responsible For Persistence Of Infection As Well As Introduction Of The Organism To Other Herds (**Newton *Et Al,* 2000)**. Differences In Virulence, Niche Adaptation, Tissue Attachment And Invasion Shared Between The Two Organisms Are Suggested To Be A Result Of Expression Of Different Proteins In *S. Equi* Versus *S. Zooepidemicus*. It Is

Hypothesized That Differences In Specificities Of Convalescent Serum Antibodies Would Identify Proteins Of *S. Equi* And *S. Zooepidemicus* Uniquely Expressed In Horses With Clinical Disease Caused By Each Of The Organisms. This Information Could Potentially Be Helpful In The Development Of New Diagnostic Tools, And Reveal Particularities In Antibody Specificities Of The Organ Systems Involved During Infection (**Verheyen *Et Al.*, 2000**).

Therefore, This Study Was Planned To Fulfill Bacteriological and Molecular Studies on *Streptococcus* Species in Horses through the following steps

1- Isolation Of Streptococcus Spp. From Horses Suffering From Strangles Signs .

2- Biochemical Identification Of Recovered Isolates

3- Serological Identification Of Recovered Isolates

3- Molecular Identification Of S.Equi And S.Zooepidemicus By Using Specific Primers .

4- Antibiogram For Recovered S.Equi And S.Zooepidemicus Isolates .

5- Detection Of Antibacterial Effect Of (Cinnamon, Anise, And Oreganium) Essential Oils On S.Equi And S.Zooepidemicus.

2. Review Of Literature

2.1. Nomenclature

The Term *Streptococcus Equi* Was First Used By Sands And Jensen At Their 1888 Description Of The Pathogen Of Strangles. Lancefield's Serotyping Scheme Was Cited In Bergey's Manual Of Determinative Bacteriology (**Breed *Et Al.*, 1948**). The Accepted Descriptive Term For *S. Equi* Was *Streptococcus Equi* (**Sands And Jensen, 1888**); For Pyogenes A Streptococci Was *Streptococcus Zooepidemicus* (**Frost And Engelbrecht, 1940**) And For Pyogenes B Streptococci Was *Streptococcus Equisimilis* (**Frost And Engelbrecht, 1940**). **Farrow And Collins In 1984** Demonstrated 92% DNA-DNA Homology Between *S.Equi* And *S. Zooepidemicus* And So They Were Reclassified As A Single Genomospecies. *Streptococcus Zooepidemicus* Was Renamed *S. Equi* Subsp. *Zooepidemicus. Streptococcus Equi* Was Designated *S. Equi* Subsp. Equi. (**Euzeby, 2004**) According To Rule 46 Of The International Code Of Nomenclature (**Lapage *Et Al.*, 1992**). Recent Use Of Techniques In Molecular Biology In Particular Multi Locus Enzyme Electrosphoresis (MLEE) And Multi Locus Sequence Typing (MLST) Provided Evidence That *S. Equi* Is A Clonal Derivative Of *S. Zooepidemicus* (**Jorm *Et Al.*, 1994; Webb *Et Al.*, 2008**). Consequently, The Correct Nomenclature Should Be *S. Zooepidemicus* Subspecies *Zooepidemicus* And *S. Zooepidemicus* Subspecies *Equi*. Unfortunately, Errors In Assigning The