شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم

HANAA ALY

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قسم

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Epidemiological Studies on Bovine Mastitis in Egypt

A Thesis Presented By

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ForThe Degree of PhD in Veterinary Medical Sciences,
(Infectious Diseases)

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Abstract

The main aim of this study is to investigate and understand the molecular epidemiology of the most common pathogens (*E coli, Staphylococcus aureus, Streptococcus uberis and staphylococcus haemolyticus*) causing bovine mastitis in Egypt dairies**,** quantify and determine the prevalence of *Salmonella* Typhimurium and *Staphylococcus aureus* in the BTM, identifying the most important risk factors affecting the level of *Staphylococcus aureus*, *Salmonella* Typhimurium , SPC and SCC, in BTM in cattle Dairies**,** apply bioinformatics approach to explore more information about *E coli TraT, Staphylococcus aureus hlg, Streptococcus uberis 16SrRNA, Staphylococcus haemolyticus tuf* and Salmonella Typhimurium *SopB* proteins , applying Insilco analyses and computational approach for designing of *TraT hlg* , *SopB* B and T cells peptide based vaccines for controlling of *Staphylococcus aureus*, *E coli* mastitis and *Salmonella* Typhimurium infection in Cattle dairies and at last quantify and assess the annual herd economic losses caused by bovine clinical and SCM within the given large Egyptian dairy herds. The cow level prevalence of cattle mastitis was 57.1% (240/420). The cow level prevalence of clinical mastitis were (19%) (80/420). The cow level prevalence of sub-clinical mastitis based on CMT and SCC were (25.5%) (107/420) (160/420) 38% respectively. Out of 220 (160 sub-clinical and 60 active clinical mastitis) positive milk samples were collected from 20 cattle dairy farms 11, 18, 40 and 19 locally field isolates were detected and confirmed phenotypic by culturing, gram staining, biochemical and molecular identification to be in overall cow level prevalence of *Staphylococcus aureus* 11 (5%), *Staphylococcus haemolyticus* 18 (8.1%), *E coli* 40 (18.1%) and *Streptococcus uberis* 19 (8.6%). PCR identification of *hlg* gene of *Staphylococcus aureus*, *E coli TraT, Streptococcus uberis 16SrRNA, Staphylococcus haemolyticus tuf* genes isolates revealed *TraT* gene was found in all forty (100%) *E coli* isolates, (*tuf)* virulence gene was found in all (18) *Staphylococcus haemolyticus* isolates (100%). (*hlg*) gene was found in 11 (42.3%) *Staphylococcus aureus* isolates and (*16SrRNA*) gene was conserved in all *Streptococcus uberis* isolates. Out of total number of 150 pooled tank milk samples were collected from 150 cattle dairy farms, 13 locally field *Staphylococcus aureus* isolates were detected and confirmed phenotypical by culturing, gram staining, biochemical and molecular identification to be *Staphylococcus aureus* in overall herd by prevalence of (8.6%). Isolation and identification of *Salmonella* Typhimurium field isolates from bulk tank milk samples revealed that 20 locally field isolates were detected and confirmed phenotypical, biochemical and molecular identification to be *Salmonella* Typhimurium in overall herd by prevalence of (13.3%). The results of total bacterial plate count (cfu/ml) revealed that the geometric mean of 150 dairy farms was 3.2×10^8 cfu/ml. The geometric mean of somatic cell count (SCC)/ml in Bulk tank milk samples of 150 cattle dairy farms were 556.7×10^3. The geometric mean value of *Staphylococcus aureus* count in this study was 3.7×10^3 cfu/ml. Serological identification of the 20 isolates revealed that they were *Salmonella* Typhimurium .The study provided various risk factors that had a clear and effective role in determining level of *E coli, Staphylococcus aureus, Streptococcus uberis and staphylococcus haemolyticus*) causing bovine mastitis in Egypt dairies, *Salmonella* Typhimurium, *Staphylococcus aureus*, Standard plate count and Somatic cell count in bulk tank milk. PCR amplification with (*hlg*) gene specific primers revealed a product with approximate size of 937 bp. (*hlg*) gene was found in 13 (54%) *Staphylococcus aureus* isolates. PCR identification of *sopB* (*SigD*) virulence gene for *Salmonella* Typhimuriym revealed a product with approximate size of 517 bp. *SopB* gene was found in all *Salmonella* Typhimurium isolates (100%). Putative domain analyses of *TraT* , *hlg* and *Tuf* and *SopB* proteins showed all conserved domains.ORF analysis of *hlg* gene sequence of *Staphylococcus aureus*, *E coli TraT, Streptococcus uberis16SrRNA, Staphylococcus haemolyticus tuf* and Salmonella Typhimurium *SopB* genes were performed showed all and complete ORFs found. On phylogenetic analysis, showed clear clustering of isolated *E coli, Staphylococcus haemolyticus, Staphylococcus aureus, Streptococcus uberis and Salmonella Typhimurium Egyptian* strains sand different strains uploaded from gene bank. The method used for estimation of mastitis economic losses in this study is highly adaptable to individual cattle farms and had a great role for assessment of specific control and management measures. The concepts described in this study help to improve our understanding of the full economic impact of cattle clinical and subclinical mastitis in Egyptian dairies. Bioinformatics analyses showed that TraT, *hlg* and *SopB* proteins were useful as vaccine candidate against E coli, *Staphylococcus aureus* mastitis and *Salmonella* Typhimurium infection in cattle dairies .The methodological approach and results from this study had a great role for facilitating of selection of useful control measures and candidates antigenic proteins for controlling of cattle mastitis in Egypt dairies.

Keywords: Bioinformatics, Economic losses, Mastitis, peptide vaccines, Risk factors**.**

Dedicated to:

My father

my mother

my wife

my son

and

my daughter

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