



شبكة المعلومات الجامعية  
التوثيق الإلكتروني والميكرو فيلم

# بسم الله الرحمن الرحيم



**HANAA ALY**



شبكة المعلومات الجامعية  
التوثيق الإلكتروني والميكروفيلم



# شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم



**HANAA ALY**



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التوثيق الإلكتروني والميكروفيلم

# جامعة عين شمس التوثيق الإلكتروني والميكروفيلم

## قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها  
علي هذه الأقراص المدمجة قد أعدت دون أية تغييرات



## يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



**HANAA ALY**



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



## **Advanced Studies on Salmonellosis in Sheep and Goats**

***Thesis presented by***

**Manar Magdy Farouk Sayed Abd El-Shafy**  
(B.V.Sc., 2013, M.V.Sc. 2017)  
**Faculty of Veterinary Medicine Cairo University**  
**For degree of Ph.D. (Infectious Diseases)**

***Under The Supervision of***

**Prof. Dr. Amal Abd El-Aziz El-Molla**

Professor of Infectious Diseases

Department of Medicine and Infectious Diseases

Faculty of Veterinary Medicine - Cairo University

**Prof. Dr. Fayez Awad-Allah Salib**

Professor of Infectious Diseases

Department of Medicine and Infectious Diseases

Faculty of Veterinary Medicine - Cairo  
University

**Dr. Yousef Adel Soliman**

Chief Researcher at Central Laboratory for  
Evaluation of Veterinary Biologics(CLEVB),

Abbasia, Cairo

**(2021)**





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



## SUPERVISION SHEET

Thesis title:

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Department of Medicine and Infectious Diseases

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Professor of Infectious Diseases

Department of Medicine and Infectious Diseases

Faculty of Veterinary Medicine - Cairo University

**Dr. Yousef Adel Soliman**

Chief Researcher at Central Laboratory for Evaluation of Veterinary Biologics (CLEVB),  
Abbasia, Cairo

(2021)





Cairo University  
Faculty of Veterinary Medicine

Department of Medicine and Infectious Diseases

- **Name:** Manar Magdy Farouk Sayed Abd El-Shafy
- **Nationality:** Egyptian
- **Degree:** PhD of veterinary science
- **Specialization:** Infectious Diseases
- **Title of thesis:** "Advanced Studies on Salmonellosis in Sheep and Goats "
- **Supervisors:**
  1. Prof. Dr. Amal Abdel-Aziz El-Molla S
  2. Prof. Dr. Fayez Awad-Allah Salib
  3. Dr. Yousef Adel Soliman

#### Abstract

Continuous emergence of antimicrobial resistant (AMR) salmonellosis poses a threat for treatment of infectious diseases in animals and humans. Identification of factors associated with development of AMR salmonellosis and providing alternative antimicrobials to limit this threat are of a great value. Therefore, the present study aimed to estimate the prevalence of salmonellosis in diarrheic sheep and goats belonging to Giza governorate in Egypt, characterize phenotypic antimicrobial sensitivity pattern of the isolated *Salmonella* spp. strains, and identify the management factors associated with perpetuation of *Salmonella* spp pathogen among the investigated flocks. Also, the study aimed to investigate the presence of enterotoxin (*stn*) gene in the isolated strains and its role in development of diarrhea, and to analyze the genetic relatedness between the isolated strains and other strains retrieved from GenBank based on partial codon sequence of *stn* gene in order to provide a scientific basis for the implementation of practical preventive measures. Furthermore, this study aimed to investigate the ability of silver nanoparticles, Bulgarian propolis ethanolic extract and locally prepared inactivated ISA-70 mantonide adjuvanted *S. Enteritidis* vaccine to alternate antimicrobials to control the development of AMR problem. A cross sectional (census) survey study was conducted, and a total number of 518 diarrheic sheep and goats belonging to 7 mixed flocks of sheep and goats were enrolled from which, rectal swabs were collected and subjected to culture for *Salmonella* spp. The presumptive identified *Salmonella* colonies were biochemically identified, serotyped and molecularly confirmed to be *Salmonella* spp. by amplification of *invA* gene using polymerase chain reaction (PCR) technique. The confirmed *Salmonella* spp. strains were examined for their susceptibility to 10 of the most commonly used antimicrobials using minimum inhibitory concentration (MIC) and to 11 antimicrobials using disc-diffusion method. The obtained questionnaire survey data about disease occurrence were statistically analyzed using Chi-square test. Twenty *Salmonella* spp isolates were recovered and the overall prevalence of salmonellosis among diarrheic sheep and goats was 3.86% (20/518), and the disease prevalence per each flock ranged from 0% to 7.55%. The prevalence of serovars among the investigated animals were 1.93% for *S. Mississippi*, 0.39% for each of *S. Durham* and *S. Enteritidis*, and 0.19% for each of *S. Ferruch*, *S. Paratyphi A*, *S. Allerton*, *S. Bonariensis*, *S. Kottbus*, and *S. Stanleyville*. The measured multiple antimicrobial resistance index (MAR) of the recovered strains revealed that, most of strains were identified as multidrug-resistant (MDR); 100% and 70% based on MIC and disc-diffusion results, respectively. The identified disease associated factors were absence of isolation of newly-purchased animals and isolation pen for sick animals, over the counter use of antimicrobials, lack of disinfection of feeding utensils and water troughs, and presence of rodents in the feed storage area. All 20 strains carried *stn* gene and the partial *stn* codon sequence of 9 strains were deposited on GenBank and their phylogenic analysis revealed high genetic relatedness to *Salmonella* strains from different sources. Interestingly, the emergence of MDR *Salmonella* spp. poses a threat for treatment and control of salmonellosis and calls for investigation for antimicrobial



alternatives. Therefore, silver nanoparticles (AgNPs) were investigated as a chemical alternative. AgNPs were synthesized by chemical reduction method. The synthesized AgNPs were characterized through imaging by transmission electron microscope (TEM), Fourier transform infrared spectroscopy (FTIR) analysis, and particle size distribution by dynamic light scattering (DLS) and zeta potential analyses using a nano-zetasizer. Furthermore, silver concentration was estimated by using elemental concentration of the nanoparticle solution through inductively coupled plasma atomic emission spectroscopy (ICP-AES). The *in-vitro* antimicrobial activity of AgNPs was investigated by estimation of minimum inhibitory concentration and minimum bactericidal concentration (MBC) for each strain using the microplate dilution method and tetrazolium salt reduction test to detect viability percentage. *In vivo* treatment efficacy was assessed in mice by determining the viable count of *S. Enteritidis* recovered from feces and by hematologic, biochemical and histopathologic examinations to confirm that use of AgNPs has no toxic or pathologic effects and to evaluate its ability in tissue regeneration following treatment. The results of TEM, DLS, Zeta potential and FTIR revealed typical characteristics of the synthesized AgNPs. AgNPs showed antibacterial activity against all recovered strains with MIC of  $\leq 0.02$ – $0.313 \mu\text{g/mL}$  (mean average  $0.085 \pm 0.126 \mu\text{g/mL}$ ) and MBC of  $0.078$ – $1.250 \mu\text{g/mL}$  (average  $0.508 \pm 0.315 \mu\text{g/mL}$ ). *In vivo* efficacy of AgNPs was observed by a reduction in the number of viable *S. Enteritidis* recovered from feces in an *S. Enteritidis* infected mouse model, with complete shedding stopping between treatment days 4 and 6. Hematologic, serum biochemical and histopathologic analyses proved the ability of AgNPs to suppress inflammatory reaction caused by *S. Enteritidis* infection. Regarding the second phytochemical alternative, the chemical composition of Bulgarian propolis ethanolic extract (EEP) was characterized using the gas chromatography-mass spectroscopy (GC-MS). The MIC and MBC of EEP were determined along with *in vivo* therapeutic efficacy with reference to hematobiochemical and histological analyses. The main antibacterial compounds of EEP belonged to flavonoids, aromatic acids, and esters. Antimicrobial efficacy of EEP was detected against all strains with variable bactericidal to bacteriostatic efficacy and with an MIC of  $\leq 0.012$ – $6.250 \text{ mg/mL}$  (mean  $1.294 \pm 1.557$ ) and an MBC of  $1.563$ – $12.50 \text{ mg/mL}$  (mean  $4.531 \pm 2.678$ ). A therapeutic efficacy against *S. Enteritidis* was also noticed. Hematological and serum biochemical tests have not showed any changes due to EEP-treatment effect. In infected groups, histological examination of the liver revealed degenerative changes in hepatocytes; these changes had almost disappeared in the EEP-treated group. Thus, the ability of EEP to alternate conventional antimicrobials in both *vitro* and *vivo* was observed. Finally, an inactivated mantonide ISA-70 adjuvanted vaccine was prepared from field strain of *S. Enteritidis* as a biological alternative. The vaccine potency was evaluated using enzyme linked immune sorbent assay (ELISA). The vaccine achieved protection 88.88%, 77.77% and 55.55% against *S. Enteritidis*, *S. Mississippi* and *S. Paratyphi A* in mice. Furthermore, the prepared vaccine achieved an increase in antibody titer in the vaccinated sheep. In conclusion, the present study paved the way towards the control of antimicrobial resistant salmonellosis by elucidating the factors associated with its development and providing safe and effective antimicrobial alternatives.

**Keywords:**

AgNPs, DLS, EEP, FTIR, goats, *invA*, MBC, MDR, MIC, PCR, phylogeny, risk factors, *Salmonella*, sheep, *stn*, TEM, vaccine.



## *Dedication*

*I dedicate this work to my parents, my sisters & my brother whom I am indebted to them for happiness in my life.*



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