

Name of Candidate: Ahmed Salah Hassan

Degree: B. SC. Faculty of Agriculture, Cairo University

Title of Thesis: Application of Recent Technology in the taxonomy of Certain Acarines in Egypt

Supervisors: Prof. Dr. Kawther M. El Kammah and Prof. Dr. Shahira O. EL Bshlawy

Department: Agricultural Zoology and Nematology

Branch: Acarology

Approval: Prof. Dr. Mohamed Abd Aziz zaher, Prof. Dr. Kawther M. El Kammah, Prof. Dr. Shahira O. EL Bshlawy, and Associate Prof. Makram Ahmed sayed.

ABSTRACT

Ticks and mites are responsible for serious economic loss, its parasitic on the animals and birds, identification of ticks and mites species has always been based on morphological characters, this method does not applied in cause of any damage in any specimen OR engorged female, Recently, molecular genetics studies were introduced to differentiate among different genera and/or species depending on DNA analysis.

- Collected ticks (*Rhipicephalus sanguineus*, *Argas persicus* and *A. arboreus*) from (dogs, fowl houses and inhabited herons), respectively.
- Collected mites (*Psoroptes natalensis*, *P. ovis*, *P. cuniculi* and *Dermanyssus gallinae*) from (buffalos, sheep, rabbits and fowl houses), respectively.
- Microscopic morphological characters to differentiate between the three species *Psoroptes cuniculi* (Delafond, 1859). *P. ovis* (Hering, 1838) and *P. natalensis* (Hirst, 1919).
- Effects of *P. cuniculi* infection on host histopathological changes cause dematites to rabbits.
- To assess the possibility of using molecular markers and five primers to identify the tick species: *Argas arboreus* (Hoogstroal&Kohls), *Argas persicus* (Oken), and *Rhipicephalus sanguineus* (Larteill), and the mites species: *Psoroptes cuniculi*, *P. natalensis*, and *Dermanyssus gallinae* based on RAPD-PCR and to estimate the genetic distances between them.
- The similarity coefficient percentage of three species of ticks was strangest relationship was scored between *A. arboreus* and *A. persicus* at the similarity of 52% while the lowest one was scored between *R. sanguineus* and *A. arboreus* at the similarity of 48%.
- The similarity coefficient percentage were strongest relationship was scored between *P. natalensis* and *P. cuniculi* at similarity of 46 %, while, the lowest was scored between *D. gallinae* and *P. natalensis*. The similarity of 42 % showed between the *P. cuniculi* and *D. gallinae*.

--	--

اسم الطالب : أحمد صلاح حسن
الدرجة : بكالوريوس في العلوم الزراعية- قسم مبيدات – كلية الزراعة جامعة القاهرة
عنوان الرسالة: استخدام التقنيات الحديثة في تقسيم بعض أكاروسات الحيوان في مصر
المشرفون : أ.د. كوثر محمد القماح - أ.د. شهيرة محمد أنسي البشلاوي
قسم: الحيوان و النيماتولوجيا الزراعية
فرع: الأكاروس
تاريخ منح الدرجة :

الموجز العربي

يعتبر القراد و الحلم المتطفل من أهم الآفات التي تسبب خسائر اقتصادية كبيرة لأنها تتطفل علي حيوانات المزرعة و الطيور الداجنة.
تقسيم القراد و الحلم المتطفل يعتمد علي الشكل المورفولوجي باستخدام الميكروسكوب و أضيف إلي استخدام الهندسة الوراثية باستخدام طريقة RAPD-PCR.
- تم جمع أنواع القراد من الحقل, النوع (ريبيسفالس سانجونيس- أرجس بيرسيكس- أرجس أربوريس) من (الكلاب- مزارع الدواجن- أبو قردان) علي التوالي.
- تم جمع أنواع الحلم من الحقل, النوع (زوروبتس ناتالينسيس- زوروبتس أوفيس- زوروبتس كونيكولاي- درمانيسيس جالنيا) من (الجاموس- الأغنام و الماعز- الأرانب- مزارع الدواجن) علي التوالي.
- تم استخدام الميكروسكوب في تحديد الاختلافات المورفولوجية فيما بين ثلاثة أنواع من جنس *Psoroptes* (*Psoroptes cuniculi* - *P. natalensis* – *P. ovis*) و أثبتت الدراسات اختلافات الشكل المورفولوجي بين الأنواع الثلاثة.
- تم إصابة عدد من الأرانب السليمة في المعمل بالأنوع *Psoroptes cuniculi* و التعرف علي مقدار الإصابة التي يسببها الجرب لجلد الحيوان.
- إيجاد علاقة القرابة بين ثلاثة أنواع من القراد و علاقة القرابة بين نوعين من أنواع الحلم و دراسة نوع ثالث من الحلم و ذلك بالاعتماد على RAPD-PCR . فوجد أن:
- أعلي صلة قرابة بين أنواع القراد كانت فيما بين أفراد الجنس الواحد (*Argas* *persicus* و *Argas arboreus* كانت 52%)
- أقل صلة قرابة بين أنواع القراد كانت فيما بين (*Rhipicephalus sanguineus* و *Argas arboreus* كانت 48%)
- أعلي صلة قرابة بين أنواع الحلم كانت فيما بين أفراد الجنس الواحد (*Psoroptes natalensis* و *Psoroptes cuniculi* كانت 46%)
- أقل صلة قرابة بين أنواع الحلم كانت فيما بين (*Psoroptes natalensis* و *Dermnyssus gllinaae* كانت 41%)
- كما أثبتت النتائج إمكانية الاعتماد علي تقنيات البيولوجيا الجزيئية في تصنيف الأكاروسات المختلفة المعروفة و الغير معروفة.

--	--

ACKNOWLEDGMENT

This work was carried out under the supervision of **Prof. Dr. Kawther M. El Kammah**, Professor of Acarology, Agricultural Zoology and Nematology Department, Faculty of Agriculture, Cairo University, to whom the author wishes to express his deepest gratitude and appreciation not only for her supervision, encouragement and guidance but also for editing the manuscript.

Thanks are due to **Prof. Dr. Shahira O. El Beshlawy**, Professor of Acarology, Agricultural Zoology and Nematology Department, Faculty of Agriculture, Cairo University for her direct supervision and encouragement throughout this study.

Thanks also to **Dr. Zaki El Fiky**, Associate Professor of Genetics, Agricultural Genetics Department, Faculty of Agriculture, Fayoum University, for the educational guidance he provided throughout the biochemical studies done in this thesis.

The author also extends his appreciation to all the staff members Parasitic Acarines Research Center, Faculty of Agriculture, Cairo University for all facilities provided throughout the study.

LIST OF FIGURES

	Pages
1 - Collecting <i>Rhipicephalus sanguineus</i> from domestic dogs.....	31
2 - Feeding method for all stages of <i>Rhipicephalus sanguineus</i>	32
3 - Collecting sites of <i>Argas persicus</i> and <i>Dermanyssus gallinae</i> from fowl houses.....	33
4 - Feeding method for (a)larval, (b)nymphal and adult stages of <i>Argas persicus</i> and <i>A. arboreus</i>	34
5 - Trees around the zoo garden, Giza Governorate inhabited by herons.....	35
6 - Collecting Sites of <i>Argas arboreus</i> from bark tree cracks and under the trees.....	35
7 - Buffaloes infested with <i>Psoroptes natalensis</i>	37
8 - Sheep infested with <i>Psoroptes ovis</i>	38
9 - Scrubbbbed <i>Psoroptes cuniculi</i> mites from lesions.....	39
10 - Rabbit infected with <i>Psoroptes cuniculi</i>	40
11 - <i>Psoroptes natalensis</i> : (A- Female genitalia), (B- Female anal region).....	54
12 - <i>Psoroptes ovis</i> : (A- Female genitalia), (B- Female anal region).....	55
13 - <i>Psoroptes cuniculi</i> (A- Female genitalia), (B- Female anal region).....	56
14 - the male anal plate of A- <i>Psoroptes cuniculi</i> , B- <i>Psoroptes</i> <i>ovis</i>	57
15 – Comparative morphological different between the genital and anal plates of <i>Psoroptes natalensis</i> , <i>P. ovis</i> and <i>P. cuniculi</i>	58
16 - Normal rabbit integument (epidermal) and (dermal) layers	

(20X).....	60
17 - Infested ear rabbit integument (crust)., (inflammation) and (20X).....	61
18 - Infested ear rabbit integument, (abscess) and (20X).....	62
19 - Infested ear rabbit integument, (<i>Psoroptes cuniculi</i>). (hyperkeratosis) ; (epidermal); (dermal); (mite) and (20X).....	63
20 - Infested ear rabbit integument (acanthosis); (crust) and (20X).....	64
21 - RAPD profiles of the three ticks species <i>Rhipicephalus</i> <i>sanguineus</i> , <i>Argas persicus</i> and <i>A. arboreus</i> amplified with RAPD primers, OPA-09(a), OPA-10(b), M: Molecular weight marker (1kb DNA ladder).	72
22 - RAPD profiles of the three ticks species <i>Rhipicephalus</i> <i>sanguineus</i> , <i>Argas persicus</i> and <i>A. arboreus</i> amplified with RAPD primers, OPA-13(c), OPA-14, OPA-15(d), M: Molecular weight marker (1kb DNA ladder).	73
23 - RAPD profiles of the three mites species <i>Psoroptes cuniculi</i> , <i>P. natalensis</i> , <i>Dermanyssus gallinae</i> . amplified with RAPD primers, (a) OPA-09, OPA-10, (b) OPA-13, OPA-14. and (c)OPA-15M: molecular weight marker (1kb DNA Ladder).	77

LIST OF TABELS

	Pages
1 - Sequence of the five arbitrary primers used in RAPD-PCR.....	47
2 - Electrophoretic patterns of RAPD-PCR amplified fragments detected in <i>Rhipicephalus sanguineus</i> , <i>Argas persicus</i> and <i>A. arboreus</i> , using OPA-09 primer.....	66
3 - Electrophoretic patterns of RAPD-PCR amplified fragments detected in <i>Rhipicephalus sanguineus</i> , <i>Argas persicus</i> and <i>A. arboreus</i> , using OPA-10 primer.....	67
4 - Electrophoretic patterns of RAPD-PCR amplified fragments detected in <i>Rhipicephalus sanguineus</i> , <i>Argas persicus</i> and <i>A. arboreus</i> , using OPA-13 primer.....	68
5 - Electrophoretic patterns of RAPD-PCR amplified fragments detected in <i>Rhipicephalus sanguineus</i> , <i>Argas persicus</i> and <i>A. arboreus</i> , using OPA-14 primer.....	69
6 - Electrophoretic patterns of RAPD-PCR amplified fragments detected in <i>Rhipicephalus sanguineus</i> , <i>Argas persicus</i> and <i>A. arboreus</i> , using OPA-15 primer.....	70
7 - The total number of RAPD-PCR fragment generated by a battery of 5 primers in <i>Rhipicephalus sanguineus</i> , <i>Argas persicus</i> and <i>A. arboreus</i>	71
8 - Similarity coefficient percentage among ticks species <i>Rhipicephalus sanguineus</i> , <i>Argas persicus</i> and <i>A. arboreus</i> based on RAPD-PCR	74
9 - The total number of RAPD-PCR fragments generated by a battery of 5 primers in three mites species: <i>Psoroptes cuniculi</i> , <i>P. natalensis</i> and <i>Dermanyssus gallinae</i>	76
10 - Electrophoretic patterns of RAPD-PCR amplified bands	

	detected in mites species <i>Psoroptes cuniculi</i> , <i>P.</i> <i>natalensis</i> , <i>Dermanyssus gallinae</i> , using OPA-09 primer.....	78
11 -	Electrophoretic patterns of RAPD-PCR amplified bands detected in the mites species <i>Psoroptes cuniculi</i> , <i>P.</i> <i>natalensis</i> , <i>Dermanyssus gallinae</i> , using OPA-10 primer.	79
12 -	Electrophoretic patterns of RAPD-PCR amplified bands detected in the mites species <i>Psoroptes cuniculi</i> , <i>P.</i> <i>natalensis</i> , <i>Dermanyssus gallinae</i> , using OPA-13 primer.....	80
13 -	Electrophoretic patterns of RAPD-PCR amplified bands detected in the mites species <i>Psoroptes cuniculi</i> , <i>P.</i> <i>natalensis</i> , <i>Dermanyssus gallinae</i> , using OPA-14 primer.	81
14 -	Electrophoretic patterns of RAPD-PCR amplified bands detected in the mites species <i>Psoroptes cuniculi</i> , <i>P. natalensis</i> , <i>Dermanyssus gallinae</i> , using OPA-15 primer.....	81
15 -	Similarity coefficient percentage among mites species <i>Psoroptes cuniculi</i> , <i>P. natalensis</i> , <i>Dermanyssus gallinae</i> based on RAPD-PCR	82

CONTENTS

INTRODUCTION.....	1
OBJECTIVES.....	2
LITERATURE REVIEW	
Ticks:	
- Economic importance.....	3
- Biology.....	4
Mites:	
- Economic importance.....	5
- Biology and Ecology of mites.....	8
Histopathological studies.....	9
Phylogeny:	
- Ticks DNA analysis (phylogeny).....	11
- Mites DNA analysis (phylogeny).....	15
MATERIAL AND METHODS	
Ticks:	
Collection and Colonization	
- <i>Rhipicephalus sanguineus</i> (Latreill).....	28
- <i>Argas (Persicargas) persicus</i> (Oken).....	28
- <i>Argas (Persicargas) arboreus</i> (Hoogstraal and Kohls).....	29
Identification.....	30
Mites:	
Collection:	
- <i>Psoroptes natalensis</i> (Hirst).....	36
- <i>Psoroptes ovis</i> (Hering).....	36
- <i>Psoroptes cuniculi</i> (Delafond).....	39
- <i>Dermanyssus gallinae</i> (De Geer).....	41

Identification	41
Histopathological studies.....	41
DNA Isoilation	43
- Solutions and buffers.....	43
- Extraction and purification.....	44
- Determination of the concentration and purity.....	45
- RAPD – PCR reaction.....	46
- Thermo-cycling profile and determination of the PCR products.....	48
- Preparation of agarose gel.....	48
- Data analysis used	49

RESULTS

Ticks:

- <i>Rhipicephalus sanguineus</i> .(Latreill).....	50
- <i>Argas (Persicargas) persicus</i> (Oken).....	50
- <i>Argas (Persicargas) arboreus</i> (Hoogstraal and Kohls).....	50

Mites:

- <i>Psoroptes natalensis</i> (Hirst).....	51
- <i>Psoroptes ovis</i> (Hering).....	51
- <i>Psoroptes cuniculi</i> (Delafond).....	51
- <i>Dermanyssus gallinae</i> (De Geer).....	51

Morphological studies:

- <i>Psoroptes natalensis</i> (Hirst).....	52
- <i>Psoroptes ovis</i> (Hering).....	52
- <i>Psoroptes cuniculi</i> (Delafond).....	53

Histopathological studies.....	59
---------------------------------------	-----------

Phylogeny:

The patterns of (RAPD-PCR) of ticks	65
The patterns of (RAPD-PCR)of mites.....	75

DISCUSSION.....	83
SUMMARY.....	87
REFERENCES.....	91
ARABIC SUMMARY.....	

Introduction

All ticks and most parasitic mites are obligate ectoparasites of domestic animals and birds, They are found throughout the world mostly in the tropics and subtropics but certain groups are adapted to desert and semi desert areas. Ticks and mites are responsible for serious economic loss which is brought about either directly by local injury, blood loss, injecting toxins and disease transmission to their hosts or indirectly by reducing host efficiency and production (eggs, meat, milk and milk products) by its influence on their metabolism and enzyme balance (El-Kammah *et al.* 1982).

Of all ectoparasites, Ixodida are highly specialized obligate parasites of a wide variety of terrestrial and flying vertebrates. They cause great economic losses in livestock production which include the transmission of infectious disenses from animal to human (viruses, rickettsiae, spirochaetes and certain protozoans) (*Theileria* and *Babesia*) between animals.

The identification of tick and mite species has always been based on morphological characters. These methods can not be applied to damaged or engorged specimens. Recently, molecular genetic studies were introduced to differentiate among different tick genera and/or species (El Kammah and Sayed, 1999; Poucher *et al.*, 1999). These methods depended upon protein and/or DNA finger prints.

We have applied a protocol for a simple, rapid and inexpensive method for DNA isolation from the eggs of three species of ticks, and three species of mites. The quantity and quality of extracted DNA were evaluated in comparison with a commercial kit, (and quality of DNA assessed by restriction endonuclease digestion and polymerase chain reaction (PCR) amplification).

The Genus *Psoroptes* Gravis, 1841 is a cosmopolitan obligate ectoparasite, which causes a debilitating dermatitis involving hair or wool loss and scab formation. The rabbit ear mite *P. cuniculi* (Delafond) is a parasite commonly found in a laboratory rabbit colonies. *P. ovis* (Hering) and *P. natalensis* (Hirst) cause a highly contagious disease of sheep and cattle, respectively. Lesions may occur on any part of the body but in badly affected animals they are most obvious on their sides. However it has been difficult to separate these species morphologically. This research is an attempt to report some morphological differences between these species, as well as DNA analysis to determine the phylogenetic origins of these species is done.

Objectives:

- 1 - Microscopic morphological characters to differentiate between the three species *Psoroptes cuniculi* (Delafond, 1859). *P. ovis* (Hering, 1838) and *P. natalensis* (Hirst, 1919).
- 2 - Effects of *P. cuniculi* infection on host histopathological changes.
- 3 - To assess the possibility of using molecular markers to identify the tick species: *Argas arboreus* (Hoogstroal&Kohls), *Argas persicus* (Oken), and *Rhipicephalus sanguineus* (Larteill), and the mites species: *Psoroptes cuniculi*, *P. natalensis*, and *Dermanyssus gallinae* (De Geer). based on RAPD-PCR and to estimate the genetic distances between them.

LITERATURE REVIEW

Tick

1- Economic importance:

The role of *Argas persicus* and *A. hermanni* in transmitting *Borrelia anserine* in chickens and pigeons respectively was observed by Madbouly *et al.* (1990).

Argas (Persicargas) persicus collected from chicken farms in Giza, Egypt was found to be more abundant in spring than summer, decreasing in the autumn. The lowest number of ticks collected was in winter. *A. persicus* was also found on turkeys, and *A. hermanni* on pigeons. The highest density of *A. persicus* and *A. hermanni* was found in spring and summer, respectively, Oyouun (1981), Oyouun *et al.* (1990).

Infested resident and migratory birds were examined and 400 ticks were collected. 8 tick species were identified, *Ixodes ricinus*, *I. Pari*, *I. arboricola*, *I. Caledonicus sculpturatus*, *Haemaphysalis punctata*, *H. erinacei erinacei* and *Hyalomma marginatum*. *Argas reflexus* was numerous in abandoned pigeon nests, Tovornik. (1990)

Parasite fauna in poultry farms was studied in Serbia. Of the total 6937 chickens, 4 geese and 6 turkeys examined, *Argas persicus* was the only tick found, Pavlovic and Nesic (1991).

The ticks on farm animals in Libya were surveyed, of which 13 species of ixodid ticks and two species of argasid ticks, *Argas persicus* and *Ornithodoros foleyi*, were collected, Gabaj *et al.* (1992).

Ixodid and argasid ticks were investigated on some mammals and birds in Belgrade. *Ixodes ricinus*, *Rhipicephalus sanguineus*, *R. bursa*,