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General overview highlights the endosymbiont -pathogen interface within ticks, focusing on Midichloria mitochondrii as endosymbiotc bacteria in Ixodes ricinus.

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

فَتَعَٰلَى ٱللَّهُ ٱلْمَلِكُ ٱلْحَقُّ ۗ وَلَا تَعْجَلْ بِٱلْقُرْءَانِ مِن قَبْلِ أَن يُقْضَىٰٓ
إِلَيْكَ وَحْيُهُۥ ۖ وَقُل رَّبِّ زِدْنِى عِلْمًا ﴿١١٤﴾

صَدَقَ اللهُ العَظِيمُ

من سورة طه

الاهداء

إلى صاحب السيرة العطرة، والفكر المُستنير؛

فلقد كان له الفضل الأوَّل في بلوغي التعليم العالي

(والدي الحبيب)، أطال الله في عُمره.

إلى من وضعتني على طريق الحياة، وراعتني حتى صرت كبيرًا

(أمي الغالية)، طيَّب الله ثراها.

إلى إخوتي؛ من كان لهم بالغ الأثر في كثير من العقبات والصعاب.

الى من افتخر بها مشرفتي الدكتوره : الاء محمد عبد الرزاق الخفاجي

إلى جميع أساتذتي الكرام؛ ممن لم يتوانوا في مد يد العون لي

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Certificate of Supervisor

I certify that the project entitled (General overview highlights the endosymbiont -pathogen interface within ticks, focusing on Midichloria mitochondrii as endosymbiotc bacteria in Ixodes ricinus) was prepared by Diaa hussein under my supervision at the College of Veterinary Medicine / University of Al-Qadisiyah.

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-- / -- / 2020

Certificate of Department

We certify that ---------- has finished his/her Graduation Project entitled (----------) and candidate it for debating.

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Abstract

Regardless of the fact that ticks can carry both non-pathological and pathological microflora, however little understood about how the microflora variability in ticks influences pathogen transmission.

Understanding the evolution of tick-borne diseases, both new and old, and their regulation requires research into the discovery of tick-pathogen and tick-endosymbiont interactions that affect vector competence and pathogen transmission. Various tick species can spread a variety of infections to animals, and also humans, including bacteria, protozoa and viruses.

Identifying the symbiont- pathogen interface in the tick vector has not been discovered expressively yet, so understanding the relationship between endo-symbiotic bacteria and tick borne pathogens is crucial to reveal the impact on tick competence and pathogen transmission. focusing on *Midichloria mitochondrii* as endosymbiotc bacteria in sheep tick Ixodes ricinus and some tick-pathogens associated with tick species, has been chosen as an exemplar on host-bacterial interaction.

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Introduction and

Review and literatures

Introduction

Ticks are obligate vertebrate hosts ectoparasites that feed on the blood of their hosts. Tick bites cause a variety of consequences, including local disruption and the possibility of toxicity excreted through tick saliva, although the biggest threat comes from the viruses that ticks will spread, of which there are several. Ticks are only equivalent to mosquitoes in terms of the number and severity of human diseases they can spread, and they, like mosquitoes, have a complicated life cycle (Beninati et al., 2004).

Ticks have a bacterial symbiotic relationship with *Midichloria mitochondrii* is present in the ovarian cells of *Ixodes ricinus* ticks, both in the cytoplasm and mitochondria). *Ixodes ricinus* consider as the primary carrier of *Midichloria mitochondrii*, as well as it role in transmission the causative agent of Lyme disease, *Borellia burgdorferi* (Beninati et al., 2004).

In addition to *Borellia burgdorferi*, ticks can spread a number of causative agents, including *Anoplasma phagocytophilum*, which causes human granulocyte anaplasmosis, ticks born fever in sheep, and pastures in cattle; as well as, it causes Canine anaplasmosis that affects dogs (Beninati et al., 2004).

Ticks also transmit *Candidatus Neoehrlichia mikurensis*, which causes febrile disease in humans; and some protozoa including *Babesia* causes red water fever in cows; in addition to some viral diseases such as tick-borne encephalitis virus in humans and *Loupi* virus disease in sheep and humans (Sassera et al., 2006).

The Midichloriaceae are members of the Rickettsiales family of bacteria, which includes intracellular bacteria found in hard ticks (Ixodidae) and other arthropods. *Midichloria mitochondrii*, a symbiotic bacterium of the *Ixodes ricinus* characterized by the ability to replicate within the mitochondria (Beninati et al., 2004; Lo et al., 2006).

***Midichloria mitochondrii* is a new clade of bacteria belonging to the Rickettsiales order. This Bacteria have been found in a variety of blood-sucking arthropods, according to PCR-based screening studies published recently. *Candidatus Midichloria mitochondrii* is still the most widespread species of ticks found all over the world. This bacterium has been reported in certain species of Ixodes, such as the sheep tick Ixodes ricinus (Zhu et al., 1992).**

***Candidatus* Midichloria mitochondrii is a bacterium that was first discovered in animal mitochondria. These bacteria have been found in the cytoplasm of ovarian cells as well as in the mitochondrial intermembrane space. These bacteria**

are located within cells with the ability to penetrate the mitochondria of the hard tick *Ixodes ricinus* and some other tick spp., which is a vector for Lyme disease.

In an adult wild female Tick, the symbiont is set. The 16S rRNA isolated from *Candidatus Midichloria* spp. was genetically analyzed, and it was discovered that the symbiont It is found in females with the *Ixodes ricinus* (100%) through tick distribution, and its density was found to be lower in males (44%) depending on electronic microscopy and in situ. hybridization studies (Sassera et al., 2006)

Midichloria mitochondrii is present in female reproductive structures of ticks, where it transmits to their offspring; bacterial genetic research, implying that, in addition to maternal transport, bacteria are transmitted horizontally through the blood of arthropod hosts (Beninati et al., 2004; Lo et al., 2006).

Recent research highlight that *Midichloria mitochondrii* multiplication in mitochondrial infected cell have an impact on organelles function without interfering with the activity of the host cell (Sacchi et al. 2004).

Mitochondrial experiments in *I. ricinus* ovaries show a diverse bacterial feature: they destroy and seem to infiltrate much of the organ's mitochondria. (Lewis, 1979; Zhu et al.,

1992; Beninati et al., 2004). This makes it the only bacteria willing to enter a multicellular organism's mitochondria. Female ticks propagate spontaneously, including the parasitism of certain mitochondria. All offspring grow naturally and acquire the bacteria, and PCR assay tests for I. ricinus indicate that 100 percent of females are harboring *Midichloria mitochondrii*, however, male bacterial numbers decrease dramatically during development, according to PCR assay studies) (Lo et al., 2006; Sassera et al., 2008).

Classification

Domain: Bacteria

Phylum: Proteobacteria

Class: Alphaproteobacteria

Subclass: Rickettsidae

Order: Rickettsiales

Family: Midichloriaceae

Genus: *Midichloria*

Species: *Midichloria mitochondrii*

Characterization of *Candidatus Midichloria mitochondrii*

Midichloria mitochondrii **is a Rickettsia- like bacteria; endosymbiont bacteria that arrange in the alpha-proteobacterial cells. It is considering relatively as new clade, as well as it appears to comprise the genera *Ehrlichia*; *Anaplasma, Wolbachia* and *Neorickettsia*. *Midichloria mitochondrii* was identified for the first time in the European sheep tick *Ixodes ricinus* (Lewis, 1979).**

According to scientific research, *Candidatus Midichloria* has been is observed by electronic microscopy as a Gram-negative genus of nonspore-forming bacteria with a bacillus shape of 0.45 m diameter and 1.2 m length (zhu et al. 1992). In general, Midichloria species are symbionts of many hard tick species (e.g., *Ixodes ricinus* and *Ixodes uriae* of the Ixodidae family). Those that live in the ovarian cells of female ticks of this genus. These bacteria were discovered in the mitochondria of the infected cells including different ovarian cells such as funicular cells and luminal cells of adult females, it has been identified in primordial cells of *Ixodes ricinus* female nymphs and larvae (Sassera et al., 2006; beninati et al. 2004).

The interaction between *Candidatus Midichloria mitochondrii* and Ticks

The importance of endosymbiont microorganisms from arthropod blood vectors should be emphasized based on their role in pathogen transmission.

During a tick bite, the bacteria could be injected into the human host. It is transmitted in tick saliva, raising the risk of it spreading to vertebrates, including humans, and causing disease.

The bacterium reproduces inside the organelles and can cause extensive damage to them without affecting the host cell's activity, (Sacchi et al. 2004).

Ticks harbor pathogenic and non-pathogenic microorganism Recent biological studies have highlighted the complex nature of tick microbiome using the genome sequencing and next generation sequencing as well as the proteomic analysis, that revealed the complication structure of tick microbiome involving both pathogenic and non-pathogenic (endosymbiont) bacteria (vayssier-Taussat et al. 2015).

Arround 10 genera of endosymbiotic bacteria have been identified so far including (*Rickettsiella, Francisella*-Like endosymbiont, *Midichloria, Wolbachia, Arsenophonus, Coxiella*-like endosymbiont, Cardinium, Spiroplsma, Rickettsia, and Lariskella) (Duron et al. 2017). However, some tick species such as *Ixodes ricinus* that harbours *Midichloria mitochondri*i as symbiotic bacteria, is predominant vector of other pathological agent such as *Anaplasma phagocytophilum, Borrelia burgdorferi* and *Rickettsia* in Europe (Capri et al. 2011). In addition, *Ixodes scapularis* that harbours *Rickettsia buchneri* (the endosymbiotic bacteria), is the main vector for *Anaplasma phagocytophilum* and *Borrelia burgdorferi* in the United states (Duron et al. 2017).

Endosymbiont and pathogens interaction

Lots of interest have been applied to identify the complex nature of internal tick environment involving the interaction between the pathogenic and commensal within ticks (vayssier-Taussat et al. 2015), in order to identify the potential impact of tick ability to transmit different diseases, in which would be a possible implication of such relationship and their consequences on vector competence and pathological agents transmission, consequently their impact on animal and human health (vayssier-Taussat et al. 2015). Previous research highlighted a negative relationship in *Ixodes scapularis* ticks associated with coinfection between *Rickettsial* endosymbiont and pathological *Borellia* (the causative agent of Lyme disease; in which highlighted reducing the rate of *Borellia burgdorferi* significantly when co-infected with *Rickettsial* endosymbiont (Simser et al.2001). Another example is interaction between the pathogenic *Anaplasma marginale* and endosymbiont Rickettsia bellii, which revealed inhibition of *Anaplasma* density upon high load and quality of endosymbiont Rickettsia (Gall et al. 2016). This reverse interface could be due to competitive interaction between the microorganism for space or may be for nutrient in which affect tick ability for pathogen transmission (Gall et al. 2016).

Chapter two

Recommendation

Recommendations:

Identifying tick microbiome, tick competence and pathological agent transmission is crucial to reveal pathogen-endosymbiont interaction and consequently reducing vector competence to transmit pathogen transmission in which eventually would suggest new approach to control ticks and tick borne diseases. Therefore, identifying the relationship between the endsymbiotic bacteria in Ixodes ricinus (Midichloria mitochondrii) as another area for research investigation would play an important role and highlight the possibility of manipulating tick microbiome and tick competence for pathogen transmission.

Chapter three

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