SURVEY OF *EHRLICHIA* AND *ANAPLASMA* SPECIES IN WHITE TAILED DEER AND IN TICKS BY REAL-TIME RT-PCR/PCR AND DNA SEQUENCING ANALYSIS

by

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Abstract

Ehrlichia and *Anaplasm*a species are rickettsial organisms which infect a variety of mammalian species. The organisms are transmitted from ticks and are maintained in reservoir hosts. Several pathogens have been identified in recent years as the causative agents for emerging infections in people. One of the primary reservoir hosts for the pathogens is the white tailed deer. In this study, 147 deer blood samples and 37 ticks were evaluated for the prevalence of *Ehrlichia*/*Anaplasma* species by TaqMan-based real time amplification assay and DNA sequence analysis. One hundred and thirteen (74%) samples tested positive with the *Ehrlichia/Anaplasma* genera-specific probe. Further analysis of the samples with the probes specific for human ehrlichiosis agents, *E. chaffeensis* and *E. ewingii* identified 4 (2.7%) and 7 (4.7%) positives, respectively. Test positives from 24 randomly selected samples were further evaluated by sequence analysis targeting to a 450 bp segment of 16S rRNA gene. All 24 samples were confirmed as positive for the *Ehrlichia* GA isolate # 4 (GenBank #U27104.1). DNAs from 37 pools of ticks collected from the white tailed deer were also evaluated. The TaqMan-based real time PCR assay with *Anaplasma/Ehrlichia* common probe identified 29 (78%) tick pools as positives whereas *E. chaffeensis*- and *E. ewingii*-specific probes identified three (8%) and one (3%) positives, respectively. The PCR and sequence analysis of tick samples identified Gram-negative bacteria species which included one endosymbiont of *Rickettsia* species (one tick pool), one *Alcaligenes faecalis* strain (three tick pools), five different *Pseudomonas* species (9 tick pools) and five different uncultured bacteria organisms (7 tick pools). Although the pathogenic potential of the white-tailed deer isolates of *Anaplasma* and *Ehrlichia* agents remains to be

established, their high prevalence and the presence of human ehrlichiosis pathogens in white-tailed deer is similar to earlier findings. The high prevalence of the deer isolates of *Anaplasma* and *Ehrlichia* species demonstrates the need for further assessment of the pathogenic potential of these organisms to people and domestic animals.

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CHAPTER ONE

LITERATURE REVIEW

Vector-borne diseases

The diseases which spread through the means of vectors are classified under vector-borne diseases (1). The vector-borne diseases are caused by viruses, bacteria, protozoa and helminth parasites. The name ‘vector’ is referred to an organism which transmit a pathogenic organism to a host (1). Arthropods are the most common disease vectors for vector borne diseases. Among arthropod vectors, mosquitoes and ticks are the most common vectors (1). The common process through which the vector-borne pathogens are transmitted is by blood feeding of vectors on their hosts (2). For example, mosquitoes are responsible for transmitting agents of diseases such as malaria, dengu fever, West Nile virus (WNV), while they feed on vertebrate hosts.

Many vector-borne diseases are known to have a significant impact on the health of humans for over centuries (3). For example, plague which is caused by a Gram-negative bacterium, *Yersinia pestis* killed a millions of people over centuries (5). The infected rodents and rodent fleas help in transmission of this disease causing agent from one host to another. The rate of transmission of vector-borne disease agents to their hosts depends on three different factors: the pathologic agent; its arthropod vector and vertebrate host (4).

Table 1: Examples of pathogens and their vectors

	Pathogenic organism	Vectors
Virus	West Nile Virus	Mosquitoes
	Rift Valley Fever Virus	Mosquitoes
	Yellow Fever virus	Mosquitoes
	Dengue virus	Mosquitoes
Bacteria	*Rickettsia akari*	House mouse mite
	Rickettsia mooseri	Body louse
	Rickettsia prowazokii	Body louse
	Rickettsia rickettsia	Tick
	Rickettsia typhi	Fleas
	Rickettsia tsutsugamuchi	Rat Mite
	Ehrlichia spp.	Tick
	Yersinia pestis	Fleas
	Borrelia burgdorferi	Tick
	Borrelia recurrentisa	Body louse
Protozoan	*Babesia microti*	Tick
	Babesia divergens	Tick
	Plasmodium falciparum	Mosquitoes
	Plasmodium malariae	Mosquitoes
	Plasmodium ovale	Mosquitoes
	Plasmodium vivax	Mosquitoes

	Leishmania braziliensis	Sandflies
	Leishmania chagasi	Sandflies
	Leishmania donovani	Sandflies
	Leishmania infantum	Sandflies
	Leishmania major	Sandflies
	Leishmania mexicana	Sandflies
	Leishmania tropica	Sandflies
	Trypanosoma cruzi	Kissing Bugs
	Trypanosoma brucei gambiense	Tsetse Flies
	Trypanosoma brucei rhodesiense	Tsetse Flies
Helminth	*Wuchereria bancrofti*	Mosquitoes
	Brugia malayi	Mosquitoes
	Mansonella ozzardi	Blackflies
	Onchocerca volvulus	Blackflies
	Mansonella perstans	Biting Midges
	Mansonella streptocerca	Biting Midges
	Loa loa	Tabanid Flies

The majority of vector-borne disease pathogens survive in nature in invertebrate vectors and their vertebrate hosts. Some intermediary animal hosts (generally wild animals) often serve as reservoir of infection. A vector acquires a pathogen from an infected host and transmits to another vertebrate host during its blood feeding cycle (2). Vector-borne disease pathogens depend on their hosts for their survival. Some of the pathogens undergo various modifications in different stages in their life cycle. In order to complete their life cycle, some vector-borne disease agents must be transmitted from one host to another. For example, *Plasmodium* species requires mosquito for sexual reproduction; the gametocytes will transform to gametes in mosquitoes and gametes form zygote. Zygotes then transform to sporozoites, which are transferred to vertebrate host during mosquito blood feeding on another host. There are many factors that determine the prevalence of vector-borne diseases. The abundance of vectors and reservoir hosts plays a major role in the prevalence of vector-borne diseases. Local environmental conditions, especially temperature and humidity may also influence the rate of prevalence of a pathogen in a given geographical area (4).

The patterns of a vector-borne disease occurrence cannot be predicted (4). Many researches worked on various vector-borne diseases to develop drugs, vaccines and also to establish the preventive measures against them. The military activities performed due to interferences of various countries in many parts of the world may also contribute for the emergence or re-emergence of vector borne diseases (6). Since 1970s, the incidence of vector-borne diseases in the world is mostly increasing or maintaining at constant level, but certainly not declining (3). Some of the examples include malaria, dengu, yellow fever, louse-borne typhus, plague, leishmaniasis, sleeping sickness, West Nile encephalitis, Lyme disease, Japanese encephalitis, Rift Valley fever, and Crimean-Congo hemorrhagic fever (3). The reasons for the

increased emergence of vector-borne diseases are not clear. Some of the possible causes include the development of drug resistance pathogens or vectors. Additionally, changes in climatic or social conditions such as urbanization and deforestation may also contribute for the increased documentation (3, 4).

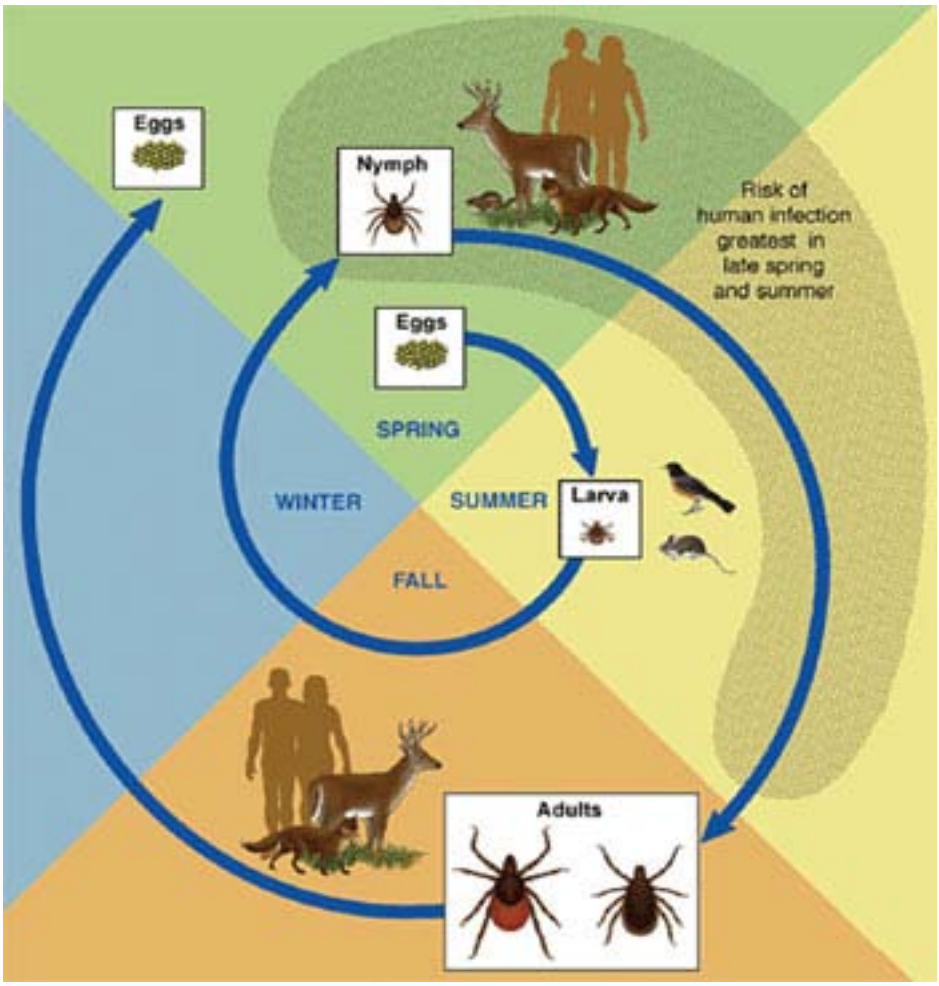
Although a great majority of the vector-borne diseases are caused by mosquito-borne pathogens, in recent years tick-borne diseases have been documented in greater numbers throughout the world (7-10). During the last three decades, many tick-borne diseases are identified as the novel and emerging diseases. They include Lyme disease (discovered in 1975) in animals and people, human macrophagic/monocytic ehrlichiosis in 1986 (66), human ewingii ehrlichiosis in 1999 (68) and human granulocytic anaplasmosis in 1993 (64). The knowledge of prevalence, transmission, survival mechanisms of tick-borne pathogens is important for developing effective methods of control and eradication.

Tick Borne Diseases:

Ticks and their role as vector:

Ticks are distributed all over the world and are responsible for the prevalence of wide range of diseases caused by viral, bacterial, and protozoan pathogens (11). Few examples are listed in Table 2. Tick-borne diseases are responsible for many serious illness in human and domestic animals (11). Ticks are ecto-parasites which depend on blood of a vertebrate host in support of

Figure 1: The general life cycle of ticks



(Source: http://www.whathealth.com/lymedisease/ticklifecycle.html)

Table 2: List of examples of ticks and the diseases that they cause in dogs and cats

Tick	Common Name	Diseases
Amblyomma americanum	Lone star tick	Canine granulocytic ehrlichiosis, Tularemia, Tick paralysis
Amblyomma maculatum	Gulf Coast tick	Hepatozoonosis
Dermacentor variabilis	American dog tick	Ehrlichiosis, Tularemia, Rocky Mountain spotted fever, Cytauxzoonosis, Tick paralysis
Dermacentor andersoni	Rocky Mountain wood tick	Rocky Mountain spotted fever, Tularemia, Cytauxzoonosis, Tick paralysis
Ixodes scapularis	Eastern black-legged tick (deer tick)	Lyme disease, Anaplasmosis, Tick paralysis
Ixodes pacificus	Western black-legged tick	Lyme disease, Anaplasmosis
Rhipicephalus sanguineus	Brown dog tick	Canine Ehrlichiosis, Rocky Mountain spotted fever, Babesiosis, Anaplasmosis, Hepatozoonosis, Haemobartonellosis

completing their life cycle. More than 850 species of ticks are known today (Furman and Loomis 1984). Ticks have 4 stages in their life cycle: egg, larva, nymph and adult (Fig.1). All stages of ticks except eggs require a blood meal for the transmission to a subsequent lifecycle stage (12). Ticks attach to vertebrate hosts by inserting their mouth parts [chelicerae (cutting mandibles) and hypostome (feeding tube)] into the host skin stay on a host until the completion of a blood meal which often takes from several hours (larvae) to several days (nymph and adult) (12). During the process of a blood meal, if a host is infected with a pathogen, it may be transferred to tick. Similarly, if a tick is infected with a pathogen acquired during a prior blood feeding stage, it may be transmitted to a naive host during its second or third blood feeding.

Tick-borne Diseases:

Tick-borne diseases are responsible for severe illnesses to humans and animals. The tick-borne disease pathogens (virus, bacteria or protozoa) not only impact the human health but also cause significant economic losses resulting from infection to domestic animals (13, 14). Some of these diseases are fatal to vertebrate hosts. This signifies the need to study about the tick-borne diseases, their causative agents, hosts and prevalence in the nature. Some of the tick-borne disease causing agents, such as *Borrelia* species (Lyme disease), *Francisella tularensis* (tularemia), *Anaplasma* species (Anaplasmosis), *Ehrlichia* species are now recognized as important emerging vector-borne pathogens worldwide (13, 15). Tick-borne diseases are documented from nearly all parts of the world (7-10).

Recently, high prevalence of several tick-borne infections have been reported in different parts of world including the countries in Europe (16, 21), Asia (17-19), Africa (20), North and South Americas. Generally, the tick-borne disease causing agents are maintained in

the nature in ticks and reservoir hosts. Their continued presence in nature, both in reservoir hosts and ticks, pose challenge to control tick-borne diseases to people and animals. Some of the tick-borne diseases, their causing agents, vectors, hosts and their prevalence are described below:

Bacteria

- Lyme disease

It is an infectious disease caused by spirochete bacteria, *Borrelia* species. The three species that cause Lyme disease are *Borrelia burgdorferi* in USA, and *Borrelia afzelii* and *Borrelia garinii* in European (22). *Borrelia* species that cause Lyme disease are also identified in various parts of world including in Africa (25-27), Asia (28-30), Australia (31), Brazil (32, 33). It was first identified in 1975 in Lyme County, Connecticut, USA (23). *Ixodes* species (ticks) serve as vectors for transmission of *Borrelia* species from one host to another. These pathogens are generally maintained in reservoir hosts such as rodents and are transmitted through ticks from one infected host to another (24). According to the CDC survey, number of human cases for Lyme disease is increasing every year in USA.

- Tularemia

Tularemia is also known as Pahvant Valley plague, rabbit fever, deer fly fever, Ohara's fever (34, 35). This infectious disease is caused by a Gram-negative bacterium *Francisella tularensis* (22). The primary vectors for *Francisella tularensis* are ticks of the genus *Dermacentor* species (22). *F. tularensis* is first

isolated and reported by George Walter McCoy in 1912 in San Francisco (36). This pathogen infects organisms such as squirrels, rodents, rabbits, dogs, sheep and humans (37). This pathogen is found mostly in two strains; Type A in USA and Type B in Europe (38).

Virus

- Tick-borne meningoencephalitis

  Tick-borne encephalitis virus (TBEV) is a member of the genus *Flavivirus,* of the family *Flaviviridae,* the causative agent of tick-borne meningoencephalitis (39-41). The primary vectors for TBEV are ticks such as *Ixodes* species ticks (41). It was first described by an Australian physician, Shneider in 1931 (42). TBEV infects small mammals, domestic animals and humans (42). Approximately 11,000 human cases are reported in Europe and many parts of Asia, annually (40).

- Crimean-Congo hemorrhagic fever (CCHF)

  CCHF is caused by *Nairovirus* in the Bunyaviridae family (43, 44). The primary vectors of CCHF are ticks such as *Hyalomma* species and sometimes *Rhipicephalus* species (44). It was first identified in 1947 (45). CCHF pathogen infects livestock such as ruminants and ostriches, humans. Mortality rates caused by this virus are between of 15-30%. This pathogen is spread in over 30 countries across the world, primarily in countries of Asia, Europe and Africa (44).

Protozoa

- Babesiosis

  Babesiosis is caused by protozoan parasites of the genus *Babesia.* They include *Babesia microti* (humans in USA), *B. canis rossi* (Dogs in Africa), *B. canis canis* (dogs in the mediterranean region), *B. ovis* (sheeps, goats in Africa, Asia, and Europe), *B. bovis* and *B. bigemina* (cattle in Southern Africa and North America). The primary vectors for this pathogen are *Ixodes* species but also can be transmitted by *Hyalomma* and *Rhipicephalus* species (46). The first *Babesia* species is identified in 1888 in Rumania (55). White-footed mouse acts as one of the reservoir host for this pathogen (49). Human babesiosis is uncommon, but recently the number of reported cases is increasing (48). *Babesia* species also identified in several other parts of the world (47).

- Cytauxzoonosis

  This tick-borne disease is caused by a protozoan hemoparasite, *Cytauxzoon felis* (50). *Dermacentor variabilis* (American Dog Tick) serves as the primary vector for this pathogen (50, 51). Cytauxzoonosis is one of the fatal diseases for cats, which has nearly 95% mortality rate (51, 52). It was first recognized in 1973 in USA (52).

Toxin

- Tick paralysis

  This is a unique tick associated disease, caused by the release of a neurotoxin produced from tick salivary glands (54). *Dermacentor andersoni* and *D. variabilis* are the two most common ticks that cause tick paralysis. However, more than 43 species of ticks are also reported to cause tick paralysis (53).

Tick-borne diseases in vertebrate animals including humans are a major problem due to their importance to the human health and economic losses resulting from infections to agricultural animals. Heartwater disease caused by a tick-borne pathogen, *Ehrlchia ruminantium* in cattle in sub Saharan Africa and Caribbean can result up to more than 90% mortality (56). For several newly discovered vector-borne diseases, few drugs are available commercially to control infections. For example, *E. chaffeensis* infection in people is treated with only doxycycline and its derivatives. Doxycycline is also a drug of choice for Lyme disease (23). Despite the treatment, many patients are not completely be cured from the pathogen or symptoms resulting from it for long periods of time. Similarly, several recent studies suggest that antibiotic treatment with doxycycline for canine ehrlichiosis (*Ehrlichia canis* infection) appears to not completely eliminate the bacterium. Vaccines are available to control some vector-borne disease causing pathogens, but may not be freely available. Vaccines also appear to lose effectiveness in preventing the vector-borne diseases, primarily due to changes like antigenic and genetic variations in a pathogen which helps the pathogens to survive in both vector and hosts.

Several new tick-borne diseases have been discovered during the last three decades and are classified as emerging diseases. Even though the adequate knowledge exists about tick-borne pathogens such as, their mode of transmission, vectors and hosts, symptoms in infected hosts and

the availability of suitable methods to diagnose, the pathogens are still prevailing in the nature and continue to cause diseases in vertebrates. It is very difficult to control tick-borne diseases because they are not contained to a specific area where they are originally reported. The diseases may spread to different areas mainly because of two factors; the spread of ticks and reservoir hosts to different geographic locations. Moreover, controlling ticks and reservoir hosts from nature is much more challenging.

Ticks are small, obligatory parasites which require blood meal from vertebrate hosts for completing their life cycle. They play a major role in the prevalence of tick-borne diseases. Ticks acquire pathogens from an infected host and transmit to another host when they are blood feeding. The pathogens can present in different life cycle stages of ticks in two ways; transtadially (from larva to nymph or nymph to adult ticks) and transovarially (from adult female tick to eggs and then to larvae). Although ticks do not have the ability to migrate to large distances, they may be transported to different places with the help of their vertebrate hosts resulting in the spread of ticks and tick-borne illnesses to different geographic locations. Birds (especially migratory birds) are also responsible in spreading of ticks because immature stages of ticks feed on them. The migration of infected ticks to different parts of the world creates an opportunity for them to feed on new hosts and may result in the spread of tick-borne diseases to new geographic locations. Most of the vector-borne pathogens and several non-pathogenic organisms may co-exist in ticks as well as in vertebrate hosts. If an infected tick is taking a blood meal, there is high probability that a vertebrate host can be infected with more than one pathogenic and non-pathogenic species transmitted from a tick. Therefore, the infected tick feeding on a naïve host may result in the spread of multiple tick- borne diseases. Many measures have been established to control tick burden to domestic animals. Acaricides are often used to

control tick burden on domestic animals and also on people. However, control of ticks from wild life is much more challenging. Thus, the wildlife serves as a constant source of ticks, including ticks with pathogens, to serve as ecto-parasites to animals and humans and also to transmit tick-borne pathogens.

Reservoir host of a pathogen is the term given to a host which harbors a pathogen, but shows no ill effects and serves as a source of infection to vectors such as to ticks and mosquitoes. Reservoir hosts play a vital role in the survival, prevalence and emergence of tick-borne diseases. Usually, wild animals and birds serve as reservoir hosts for various tick-borne pathogens. These are the main source of the tick-borne pathogens from which, the infected ticks get infection and transmit to other hosts, such as domestic animals and humans. The presence of the pathogens among vertebrate hosts may also contribute to the rate of their prevalence in an area. Sometimes, the infected reservoir hosts are transported from one country to another and may contribute to spreading the disease causing agents to new geographic locations. The reservoir hosts may include both large and small mammals such as white-tailed deer, squirrels, rodents and mice, all of which contribute to the spread of the tick-borne diseases. It is highly difficult to control the prevalence of tick-borne pathogens among the reservoir hosts as most of them are wild animals.

Apart from aiding in prevalence and survival of pathogens, reservoir hosts and ticks may also contribute to the origin of novel strains and species of pathogens. It is known that most of the pathogenic and non-pathogenic species, including bacteria, viruses and protozoans co-exists in ticks and vertebrate hosts. Likewise, the gut of human or animals is known to contain numerous bacteria and protozoans; and some of them exchange genetic material among them for their survival in host (58). Generally, most of the organisms undergo some modifications both

genetically and physiologically for adapting to their host environments for their survival. Micro-organisms co-existing within a host or a tick may also lead to altered pathogenic potential. Similarly, co-existence of closely related species within a vector or vertebrate host may lead to the exchange of genetic material among them. This may result in the emergence of novel species or strains with altered pathogenicity and adaptations to new hosts. Some of the bacterial microorganisms discovered in recent years in ticks may or may not be pathogenic to vertebrate animals. For example, *Anaplasma* species strains such as WTD 76 (GenBank: DQ007351.1), WTD 81 (GenBank: DQ007352.1), wz 57 (GenBank: AY180920.1) and *Ehrlichia* species strains such as GA isolate 4 (GenBank: U27104.1), 2 (GenBank: U27103.1) and OK isolate No. 1 (GenBank: U27102.1), 3 (GenBank: U27101.1) are reported in the literature but their pathogenic potential, however, remains to be determined. It is not clear if the recently discovered diseases caused by *Ehrlichia* and *Anaplasma* species is the result of genetic modifications to the non-pathogenic species within ticks and reservoir hosts. This hypothesis, while never been tested, is a logical one to consider because ticks and white-tailed deer (one of the important wildlife reservoir hosts) are reported to contain several non-pathogenic *Ehrlichia* and *Anaplasma* species. Human macrophagic/monocytic ehrlichiosis agent, *E.chaffeensis* was not reported as a pathogen of human and any other vertebrates until 1986 (66). Similarly, *A. phagocytophilum* infections were known for several decades as the causative agent for diseases in horses and cattle is documented for first time as the human pathogen in 1990 (64). It is possible that the pathogenic strains which are reported as the causative agents for several diseases may be non-pathogenic for some time and due to genetic transformations or due to genetic exchange in between related species, they may become pathogenic organisms. As per the literature, there are many strains and species are present in ticks and vertebrate hosts whose pathogenic potential is not yet reported.

Effective control of tick-borne diseases requires the knowledge about the pathogen prevalence in both vectors and vertebrate hosts. Periodically epidemiological surveys should be conducted for determining the rate of the prevalence of tick-borne disease causing agents in vectors and vertebrate hosts. The knowledge of prevalence of the pathogens in vectors and hosts may aid in taking the appropriate measures to control and prevent the pathogens to cause disease to human and animals. Epidemiological surveys also aid in identifying novel strains or species related to these pathogens under investigation.

Anaplasmataceae family members of the genus *Ehrlichia* and *Anaplasma* are known as agents for causing various tick-borne diseases in animals and humans which may cause severe illness (59-65, 69, 70, and 72). These are Gram-negative, obligatory intracellular pathogens (57). They include human monocytic ehrlichiosis (HME), human ewingi ehrlichiosis (HEE), and human granulocytic anaplasmosis (HGA) caused by *Ehrlichia chaffeensis, E. ewingii* and *Anaplasma phagocytophilum*, respectively. These pathogens are regarded as emerging pathogens as these documented cases have been steadily increased every year (67). These pathogens are distributed all over the world. They are successfully maintained in nature and cause various illnesses to humans and animals. Based on the host immunity, the pathogen infections can also result in fatal illness to people. Some of the pathogenic species are listed in Table. 3.

Table 3: List of some *Ehrlichia* and *Anaplasma* species and their infections in vertebrate hosts (78)

Pathogen	Vector species	Reservoir host	Host	Disease	Cell type interactions
A. marginale	*Dermacentor andersoni* (tick)	Water buffalo, Mule deer, American bison	Cattle, wild ruminants	Bovine anaplasmosis	Erythrocytes
A. phagocytophilum	*Ixodes scapularis* (tick)	White-footed mice	dogs, horses, humans	Human Granulocytic Anaplasmosis (human), Anaplasmosis (animals)	Neutrophills
E. canis	*Rhipicephalus sanguineus* (tick)	Coyotes	Dogs	Canine ehrlichiosis	Monocytes and Neutrophills
E. chaffeensis	*Amblyomma americanum* (tick)	White-tailed deer	dogs, goats, coyotes, & humans	Human Monocyte or Macrophage Ehrlichiosis	Monocytes or Macrophages
E .ewingii	*Amblyomma americanum* (tick)	White-tailed deer	Dogs, humans	Human ewingi ehrlichiosis	Neutrophills
E. ruminantium	*Amblyomma spp.* (ticks)	Antelopes	Cattle, sheep, goats, antelope	Heartwater disease	Vascular endothelial cells and reticulum cells of the lymph nodes

CHAPTER TWO

Survey of *Ehrlichia* and *Anaplasma* species in white tailed deer and ticks by real-time RT-PCR/PCR and DNA sequencing analysis

Introduction:

Ehrlichia and *Anaplasm*a species belong to the Anaplasmataceae family of the order Rickettsiales (57). They are Gram negative, obligatory and intracellular pathogens. They have a wide range of vertebrate hosts such as deer, dogs, coyotes, cattle, horse and humans (57). The organisms that are known till now are transmitted from infected ticks such as *Amblyomma* and *Ixodes* species from one host to another. They are maintained in nature in reservoir hosts and ticks. Most of the pathogens co-exist in reservoir hosts and ticks along with other species whose pathogenecity is unknown. There is a high probability that an infected host contains more than one species related to the pathogens of several genera.

Several pathogens identified recently are considered to be causative agents of emerging diseases in vertebrate hosts. They include *E. chaffeensis*, the causative agent of human monocytic ehrlichiosis (HME) identified in 1987 (66). *E. chaffeensis* also infects dogs, goats, coyotes, white-tailed deer and humans. *E. chaffeensis* invades monocytes and macrophages of vertebrate hosts. Similarly, *E. ewingii* is identified as the causative agent of human ewingi ehrlichiosis (HEE) in 1993 (70). This organism is originally identified as canine granulocytic ehrlichiosis pathogen in 1932. *A. phagocytophilum* previously known as the bovine and equine pathogen is first identified as a causative agent of human granulocytic anaplasmosis (HGA) in 1990 (64).

Ehrlichia and *Anaplasm*a species pathogens have high prevalence in several parts of the world are assessed by many epidemiological surveys done in recent years. Previous epidemiological surveys also revealed that the pathogenic organisms co-existent with other pathogenic and non-pathogenic organisms. Blood samples collected from reservoir hosts and ticks recovered from a field or from an animal are valuable in evaluating the prevalence of

infections with both pathogenic and other *Ehrlichia* and *Anaplasm*a species with unknown pathogenecity. As one of the primary reservoir hosts for the *Ehrlichia* and *Anaplasm*a species is the white tailed deer, this study utilized white tailed deer blood samples and ticks collected on the animals were used for determining the prevalence of the *Ehrlichia* and *Anaplasm*a species. The prevalence of the *Ehrlichia* and *Anaplasm*a species was assessed by using a highly sensitive and specific real time PCR/RT-PCR assays and by performing DNA sequencing analysis of the *Ehrlichia*/ *Anaplasm*a species 16S rDNA segment amplified by PCR. The results obtained from this study and their importance to the emergence of new diseases was discussed.

Materials and Methods

Deer Blood and Tick Collection: A total of 147 blood samples were collected from the hunted deer's in Kansas in November, 2009. Approximately 10 ml of blood from each deer was collected in EDTA tubes and immediately kept in ice packs for overnight shipping to Kansas State University. After receiving the samples, they were stored at 4°C and within two days, they were processed to isolate DNA or RNA. The blood samples were centrifuged at 10,000 x g for 10 min to separate plasma from blood cells. About 0.2 ml of buffy coats from each sample was then used to extract DNA or RNA. Thirty seven tick pools (each containing one or two ticks) were collected from deer. The ticks were shipped to Kansas State University and were stored at -20°C until performing DNA isolation.

DNA extraction: DNA extraction from deer blood samples was performed using a column-based QIAamp DNA mini and Blood Mini kit (QIAGEN). The kit protocol was followed as per the manufacturer's instructions using 0.2 ml of buffy coats per each sample of blood for DNA isolation. Briefly, 20 µl of proteinase K from kit was added to 0.2 ml of buffy coat of deer blood sample and vortexed. Then, 0.2 ml of Buffer AL was added and vortexed for ensuring the lysis of cells. The entire lysate mixture was incubated for 10 min at 56°C in a water bath. Then, 0.2 ml of 100 % ethanol was added and vortexed. The samples were transferred to a QIAmp Mini spin column fitted in a collecting tube and centrifuged at 6,000 x g for 1 min. The solution collected in the tube was discarded. The column was washed once each with 0.5 ml of Buffer AW1 and AW2 by spinning at 6,000 x g for 1 min and 20, 000 x g for 3 min, respectively and discarded the solution in collecting tubes. The final step included the elution of DNA from the column by adding

50 µl of Buffer AE or nuclease free distilled water and centrifuged for 1 min at 20, 000 x g. DNA solution was collected in a clean 1.5 ml tube was then stored at -20°C until use. Typically 2 µl of the extracted DNA was used for real time PCR or PCR assays.

DNA isolation from tick pools was performed by using AllPrep DNA/RNA Mini Kit (QIAGEN, Valencia, CA) and automated system Qiacube (QIAGEN). The kit protocol was followed as per the manufacturer's instructions. Briefly, up to 20 mg of tick sliced in to small pieces and placed in 350 µl Buffer RTL Plus and then processed in the Qiacube for DNA isolation. Final purified DNA was collected in 100 µl Buffer EB. Typically 2 µl of the extracted DNA was used for real time PCR or PCR assays.

RNA extraction: Trireagent BD Kit (SIGMA, Missouri, USA) was used to isolate RNA from 0.2 ml buffy coats of deer blood. The kit protocol was followed as per the manufacturer's instructions. Briefly, 0.75 ml of Trireagent BD (guanidine thiocyanate and phenol) was added to 0.2 ml of buffy coat of deer blood sample and vortexed. Then, 20 µl of 5 N acetic acid was added and vortexed. The tubes were incubated at room temperature for 5 min and 0.2 ml of chloroform was added and vortexed. Then, the samples were centrifuged at 12,000 x g for 15 min at 4°C. The clean supernatant was transferred to new tubes and the pellets were discarded. Now, 0.5 ml of chilled 100% isoprponal was added to each supernatant transferred and incubated for 10 min at room temperature after vortexing. Centrifugation was done at 12,000 x g for 8 min at 4°C. The supernatant was discarded. The pellet was rinsed with 1 ml of cold 75% ethanol. The final purified RNA pellet was resuspended in the 50 µl of TE buffer or nuclease free distilled water and stored at -20°C for further use. Typically 2 µl of the extracted RNA was used for real time RT-PCR or RT-PCR assays.

Real Time PCR and RT-PCR: RNAs and DNAs were utilized for analyzing the presence of *Ehlichia* /*Anaplasma* species by performing real time TaqMan probe based PCR and RT-PCR assay by following the methods described previously (73). The primers and probes used in this assay were listed in Table 4.The assay is designed to identify the presence of any species of *Ehlichia* or *Anaplasma* using a *Ehlichia* /*Anaplasma* genera specific TaqMan probe labeled with ROX dye. This is a triplex assay targeted also to identify the presence of nucleic acids of *E. chaffeensis* and *E. ewingii* using specific TaqMan probes labeled with TET and FAM dyes, respectively. The excitation and emission wavelength of the florescent dyes, FAM are 492 nm and 516 nm, TET are 517 nm and 538 nm and ROX are 585 nm and 610 nm, respectively. Positive controls used in this assay contain recombinant plasmids with 16S rRNAgene fragments of *E. chaffeensis* and *E. ewingii* in 1:1 ratio (73). Real Time PCR and RT-PCR was performed by using Platinum qPCR and SSIII One-step qRT-PCR Kits (INVITROGEN, California, USA), respectively. Reaction mixture (25 µl) include 12.5 µl of 2 x reaction buffer, 1.7 µl of 50 mM Mg_2SO_4, 0.5 µl of 20 µM *Ehlichia* /*Anaplasma* TaqMan forward and reverse primers, 0.5 µl each of 7.5 µM *E. chaffeensis, E. ewingii* and *Ehlichia*/*Anaplasma* common probes, 1 µl of SSIII Taq polymerase, 0.2 µl of Platinum Taq polymerase and 4.8 µl of nuclease free water per 1 sample. The thermal cycles for PCR included the initial denaturation for 3 min at 94°C, 45 cycles of 94°C for 30 sec, 52°C for 30 sec and 72°C for 60 sec, and finally one cycle of 72°C for 3 min. The cycles for RT-PCR were the same as for DNA but included an extra step of incubation at 48°C for 30 min at the beginning of temperature cycles to convert RNA to cDNA. Real Time PCR and RT-PCR were performed using a Smart Cycler (Cephid systems).

Table 4. Primers and Probes used in the Real time PCR and Real Time RT-PCR (73)

	Sequence	Length
Primers		
Ehrlichia/Anaplasma TaqMan forward primer	5' ctcagaacgaacgctgg	17
Ehrlichia/Anaplasma TaqMan reverse primer	5'catttctaatggctattcc	19
Ehrlichia/Anaplasma forward primer (RRG1)	5'caagcctaacacatgcaagtcgaac	25
Ehrlichia/Anaplasma reverse primer (RRG27)	5'gtattaccgcggctgctggcac	22
TaqMan probes		
E. chaffeensis	5'TET/cttataacctttggttataaataattgttag/BQH2*	32
E. ewingii	5'FAM/ctaaatagtctctgactatttagatagttgttag/BQH2*	34
Ehrlichia/Anaplasma common	5'ROX/taacacatgcaagtcgaacgga/BQH2*	22

PCR and RT-PCR: A subset of samples tested positive by real time PCR and RT-PCR assays were assessed by PCR assay to amplify a 450 bp fragment of 16S rRNA gene using *Ehlichia* and *Anaplasma* genus specific primer pair (73). Positive control used in this assay included recombinant plasmid with 16S rRNA partial gene segment of *E.chaffeensis* or *E. ewingii* (73). Amplification reactions were performed using AmpliTaq PCR kit (APPLIED BIOSYSTEMS, USA) and SSIII One-step qRT-PCR Kit (INVITROGEN, California, USA) for DNA and RNA samples, respectively. The 50 µl reaction mixture for DNA samples included 5 µl of 10 x reaction buffer, 3 µl of 25 mM $MgCl_2$, 1 µl of 10 µM dNTPs, 1 µl each of 10 µM *Ehlichia* /*Anaplasma* common forward primer (RRG1) and reverse primer (RRG27), 0.4 µl of AmpliTaq DNA polymerase and 37.6 µl of nuclease free water. The 50 µl reaction mixture for RNA samples included 25 µl of 2 x reaction buffer, 1 µl each of 10 µM *Ehlichia* /*Anaplasma* common forward primer (RRG1) and reverse primer (RRG27), 1 µl of SSIII DNA Taq polymerase and 20 µl of nuclease free water. The temperature cycles used for this assay are: initial heating for 3 min at 94°C, followed by 40 cycles of 94°C for 30 sec, 52°C for 30 sec, and 72°C for 45 sec, then one cycle of 72°C for 5 min and finally 4°C hold cycle. For RNA samples, an extra incubation step of 1 hr at 50°C was included prior to the initiation of above described thermal cycles. Five microlitre of PCR products were assessed by resolving on 1% agarose gel.

Gel Purification of PCR products: The selected subsets of PCR products resolved on 1% agarose gel were gel-purified by using QIAamp Gel purification kit (QIAGEN, Maryland, USA). The desired bands were excised under UV light and the gel pieces were transferred to the 1.5 ml tubes. Three volumes of QG buffer was added to the gel pieces and incubated at 50°C for 10 min for dissolving the agarose. One volume of isopropanol is added to the solution and mixed

thoroughly. The solution was transferred to the QIAamp spin column with collecting tube and centrifuged at 17,900 x g for 1 min. The solution in the collecting tube containing the melted agarose was discarded. To remove traces of agarose from the spin column, 0.5 ml of QG buffer was added to the column and centrifuged at 17,900 x g for 1 min. The solution in collecting tube was discarded. Then the pellet in column was washed with 0.75 ml of PE buffer by spinning at 17,900 x g for 1 min. The solution collected in collecting tube was discarded again. Finally, to recover DNA from the column 30 µl of Extraction buffer (E.B) was added to the column and incubated at room temperature for 30 min. The E.B solution that contains purified DNA fragments was collected in fresh 1.5 ml tube after spinning at 17,900 x g for 1 min. Five microlitre of DNA recovered from the spin column was resolved on 1% agarose gel to determine the presence and concentration. The remaining DNA solution was stored at 4°C until use.

DNA Sequencing: Above described purified PCR products were used for performing DNA sequence analysis to establish the identity of unknown *Ehlichia* and/or *Anaplasma* species. A select subset of samples was analyzed using Beckman Coulter CEQ 8000 Genetic Analysis system and DNA sequencing Quick Start Kit (BECKMAN COULTER, California, USA). Approximately, 16 ng of purified DNA templates are needed for DNA sequencing for fragments of 500 bp in length. The purified DNA products were used as templates for the sequencing analysis. The 20 µl reaction mixture included 0.5-10 µl of DNA template (about 16 ng), 0-9.5 µl of nuclease free distilled water, 8 µl of DTCS Quick start master mix, 2 µl of 2.5 µM *Ehlichia* /*Anaplasma* common forward primer (RRG1) or reverse primer (RRG27). The thermal cycles included 30 cycles of 96°C for 20 sec, 50°C for 20 sec and 60°C for 4 min. After the completion of thermal cycles, 5µl of Stop solution/Glycogen mixture (2 µl of 3 M Sodium Acetate (pH 5.2), 2

µl of 100 mM Sodium EDTA (pH 8.0), 1 µl of 20 mg/ml of glycogen) was added to each sample. Samples were vortexed briefly and 60 µl of cold 95% ethanol was added. Centrifugation was done at 20,000 x g for 15 min at 4°C. Supernatant was discarded. The white pellet with DNA was washed twice with 200 µl of chilled 70% ethanol. After each addition of 70% ethanol, the samples were centrifuged at 20,000 x g for 2 min at 4°C. The pellet was vacuum dried for 10 min and 40 µl of sample loading solution was added and applied into DNA sequencing machine for analysis. The samples were analyzed and their sequences were obtained for identification of specific organism.

BLAST Search Analysis: The sequences obtained following the DNA sequence analysis of purified PCR DNA samples were used for identifying *Ehlichia*/*Anaplasma* species. The BLAST search program available online at the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for finding matches to the DNA sequences available in the GenBank database.

Results

Real Time PCR and RT-PCR:

To evaluate the prevalence of *Ehrlichia* and *Anaplasma* species in white-tailed deer, DNA and RNA extracted from 147 blood samples were analysed by real time TaqMan probe based PCR and RT-PCR assays. The TaqMan probes and PCR primers were listed in Table 4. One hundred and thirteen samples (74%) tested positive with *Ehrlichia* and *Anaplasma* species common probe. These included 75 tested positive with both DNA and RNA templates, 16 positives for only RNA templates and 22 positives for only DNA templates.

The presence of *E. chaffeensis* and *E. ewingii* was assessed in the deer blood, and found that 147 sample nucleic acids by real-time PCR and RT-PCR assays with TaqMan probes specific to each of these two species. The analysis identified fewer positives with these probes; four *E. chaffeensis* positives and seven *E. ewingii* positives. The species identity in the remaining 102 blood samples is not clear from the PCR and RT-PCR assays. Real time PCR and RT-PCR positives of deer blood were listed in Table 5. The data were presented as Ct values; PCR cycles at which the samples tested positive by real time PCR/RT-PCR assays. *E. chaffeensis* positives included one test positive with DNA and RNA templates and another positive with DNA template alone. Similarly, two of the *E. ewingii* positives tested positive with only RNA and three samples tested positive with both DNA and RNA templates.

Fig 2. Deer blood samples tested positive with probes for *E.chaffeensis*, *E.ewingii* and with *Ehrlichia/Anaplasma* genera specific probe.

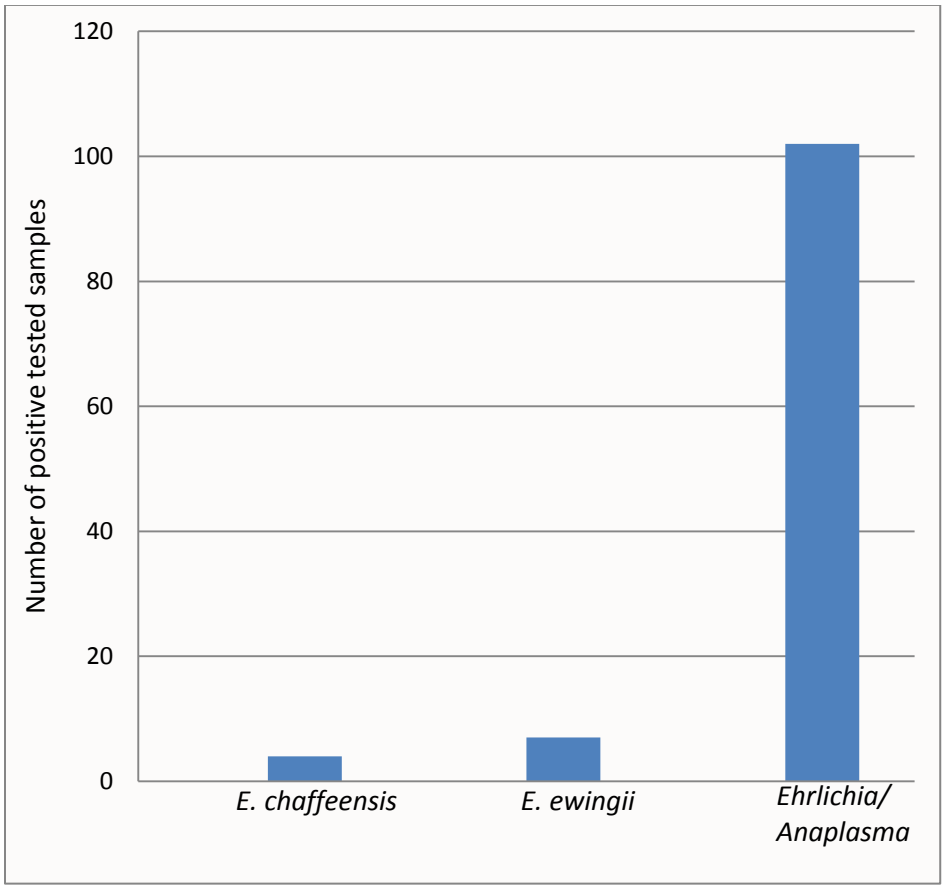


Table 5. Ct-values[a] of the blood samples given in Real time PCR and RT-PCR assays

	DNA Ct			RNA Ct		
Sample ID	Fam-E. ewingii	Tet-E. chaffeensis	Rox-Common	Fam-E. ewingii	Tet-E. chaffeensis	Rox-Common
1	0	0	21.66	0	0	23.52
2	0	0	0	0	0	24.42
3	0	0	23.6	0	0	28.74
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
9	0	0	23.35	0	0	0
10	0	0	0	0	0	0
11	0	0	0	0	0	0
14	0	0	0	0	0	0
15	0	0	0	0	0	0
19	0	0	0	0	0	0
20	0	0	0	0	0	0
21	0	0	25.78	0	0	27.51
24	0	0	26.76	0	0	0
27	0	0	19.2	0	0	22.84
30	0	0	21.59	0	0	20.07
31	0	0	22.79	0	0	23.22
34	0	0	0	0	0	26.1
37	0	0	0	0	0	26.62
39	0	0	25.08	0	0	30.22
41	0	31.62	26.16	0	0	17.41
45	0	0	0	0	0	21.19
46	0	0	0	0	0	0
47	0	0	0	0	0	0
48	0	0	19.47	0	0	25.23
53	0	0	25.41	0	0	0
54	0	0	25.09	0	0	0
55	0	0	22.63	0	0	0
59	0	0	22.73	0	0	0
63	0	0	0	29.34	0	25.36
64	0	0	22.12	0	0	29.26
65	0	0	0	0	0	0
69	0	0	0	0	0	0
73	0	0	20.15	0	0	0
76	0	0	22.85	0	0	0
80	0	0	23.33	0	0	0

81	0	0	0	0	0	23.79
82	0	0	0	0	0	27.32
83	0	0	18.68	0	0	0
85	0	0	20.93	0	0	0
86	0	0	19.54	0	0	0
92	0	0	0	0	0	0
93	0	0	0	0	0	16.77
99	0	0	26.33	0	0	0
101	0	0	0	0	0	0
102	0	0	0	0	0	0
105	0	0	0	0	0	0
107	0	0	0	0	0	21.11
111	0	0	0	0	0	0
112	0	0	0	0	0	18.86
113	0	0	23.06	0	0	27.21
116	0	0	23.37	27.65	0	24.19
118	0	0	0	0	0	21.74
121	0	0	0	0	0	19.53
123	0	0	21.57	0	0	22.51
124	0	0	0	0	0	0
125	0	0	21.51	0	0	21.41
129	0	0	0	0	0	0
131	0	0	24.19	0	0	28.79
132	0	0	0	0	0	0
133	0	0	24.14	0	0	21.94
139	0	0	26.46	0	0	29.15
144	0	0	21.31	0	0	21.68
146	0	0	20.21	0	0	24.7
147	0	0	0	0	0	0
152	0	0	21.38	0	0	22.25
154	0	0	22.55	0	0	19.45
156	0	0	24.86	0	0	26.45
157	0	0	20.24	0	0	20.61
159	0	0	16.49	0	0	15.24
160	0	0	19.73	0	0	21.65
162	0	0	23.73	0	0	25.84
163	0	0	20.41	0	0	23.23
165	0	0	25.93	0	0	27.85
166	29.98	0	25.25	0	0	28.15
167	0	0	25.12	0	0	23.83
169	0	0	17.98	0	0	19.93
172	0	28.79	17.93	0	24.97	20.24
173	0	0	23.16	27.32	0	23.63

174	0	0	22.85	0	0	28.22*
175	0	0	22.09	0	0	24.49
178	0	0	0	0	0	0
180	0	0	24.52	0	0	28.19
181	0	0	0	0	0	0
182	0	0	27.25	0	0	31.43
183	0	0	0	0	0	22.8
187	0	0	24.27	0	0	29.54
189	0	0	17.72	0	23.76	18.19
192	0	0	23.16	0	0	23.43
193	0	0	22.43	0	0	22.48
194	0	0	0	0	0	0
196	0	0	21.11	0	0	25.87
201	0	0	0	0	0	26.2
202	0	0	21.9	0	0	19.42
206	0	0	21.66	0	0	20.76
207	0	0	21.81	0	0	24.87
208	0	0	25.75	0	0	0
210	0	0	0	0	0	0
211	0	0	24.19	0	0	0
212	0	0	20.97	0	0	21.09
213	0	0	18.54	0	0	20.5
216	0	0	23.34	0	0	22.1
217	0	0	0	0	0	0
219	0	0	0	0	0	29.14
220	30.13	0	15.91	28.15	0	19.28
221	0	0	22.17	0	0	26.25
225	0	0	0	0	0	0
226	0	0	0	0	0	0
229	0	0	0	0	0	0
230	0	0	0	0	0	19.49
231	0	0	20.2	0	0	22.2
234	0	0	26.16	0	0	25.8
236	0	0	27.86	0	0	0
237	0	0	24.77	0	0	30.28
238	0	0	23.33	0	0	21.36
240	0	0	21.26	0	0	21.06
241	0	0	0	0	0	26.13
242	0	0	20.41	0	0	21.34
245	0	0	29.11	0	0	28.43
249	0	0	0	0	0	25.96
252	0	0	20.29	0	0	22.19
253	0	0	24.34	0	0	26.96

257	0	0	22.79	0	0	22.55
263	0	0	17.69	0	0	15.74
265	0	0	21.87	0	0	19.24
269	0	0	27.46	0	0	0
271	0	26.10	23.98	0	0	27.90
272	0	0	22.14	0	0	19.29
274	0	0	23.65	0	0	19.7
276	0	0	19.8	0	0	22.23
277	0	0	25.26	0	0	0
280	0	0	23.15	0	0	21.43
283	0	0	0	0	0	0
287	0	0	23.78	0	0	27.28
289	0	0	22.06	0	0	21.89
290	0	0	22.35	0	0	23.06
292	0	0	20.34	0	0	17.54
294	0	0	0	0	0	0
297	0	0	23.12	0	0	19.98
299	0	0	26.7	0	0	25.87
302	0	0	0	27.76	0	21.49
305	0	0	22.12	0	0	19.36
308	0	0	19.83	0	0	20.47
310	0	0	23.35	0	0	25.73
311	28.15	0	22.3	26.59	0	24.06
313	0	0	25.75	0	0	24.66

*Ct-value refers to the PCR cycle at which the fluorescence crosses 10 units for each fluorescence emission channel.

The presence of *Ehrlichia* and *Anaplasma* species DNA in ticks was also evaluated by real time TaqMan probe based PCR assay. The DNA extracted from the 37 pools of ticks (each pool has one or two ticks) used as templates for this assay. Twenty nine of these samples (78%) tested positive with *Ehrlichia /Anaplasma* common probe. Further analysis was performed to identify the presence of *E. chaffeensis* and *E. ewingii* DNA in ticks using these two species specific TaqMan probes. Only three (8%) of 29 pools were confirmed as positives for *E. chaffeensis*, and one (3%) as the positive for *E. ewingii*. The Ct-values of all samples tested positives by real time PCR and RT-PCR were listed in Table 6. The positives identified for *E. chaffeensis* and *E. ewingii* are similar in number compared to deer samples tested positives for these two species.

PCR or RT-PCR:

To determine the identity of the highly prevalent *Ehrlichia* and *Anaplasma* species in deer blood samples and ticks, the nucleic acid templates were used to amplify a segment of 16S rRNA. The amplicons that tested positive by PCR (as judged from the presence of a predicted fragment identify on a 1% agarose gel) were used for DNA sequencing analysis were performed on a subset of samples. PCR or RT-PCR were performed using a primer set that is expected to amplify 450 bp segment of 16S rRNA gene from any known *Ehrlichia* and *Anaplasma* species (the primers used for these analysis were listed in Table 1). Twenty six samples out of 113 deer blood nucleic acids (DNA or RNA) analyzed yielded predicted PCR positive amplicons as assessed after resolving on 1% agarose gels (Fig. 4). Similar analysis on 37 tick DNA samples resulted in 33 PCR positives (Fig. 5). The PCR positives are considerably more for tick DNA templates compared to deer blood nucleic acids.

Fig 3. Tick samples tested positive with probes for *E. chaffeensis, E. ewingii* and with *Ehrlichia/Anaplasma* genera specific.

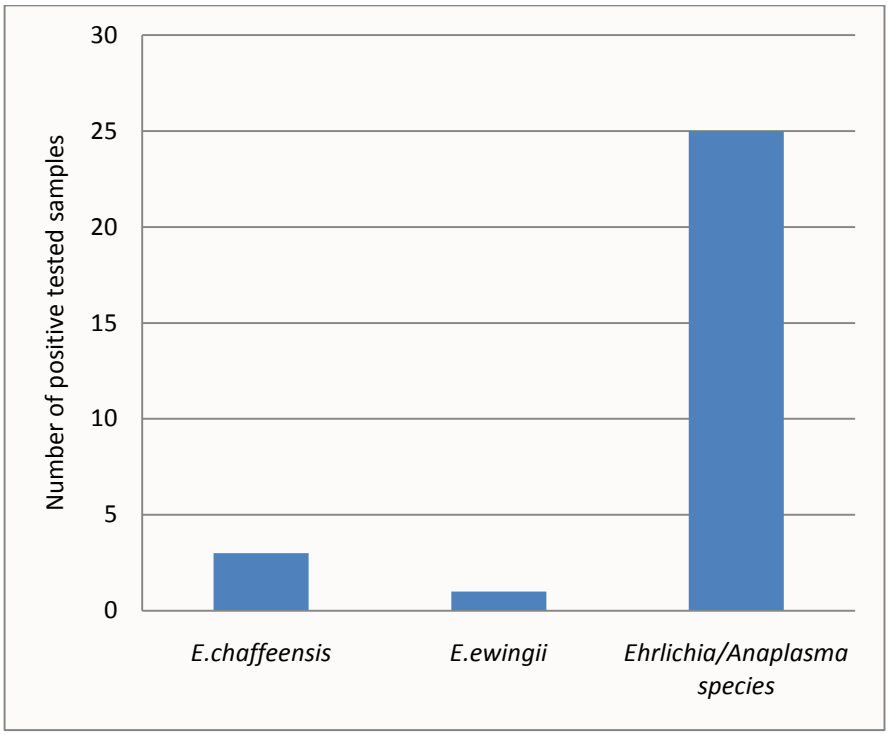
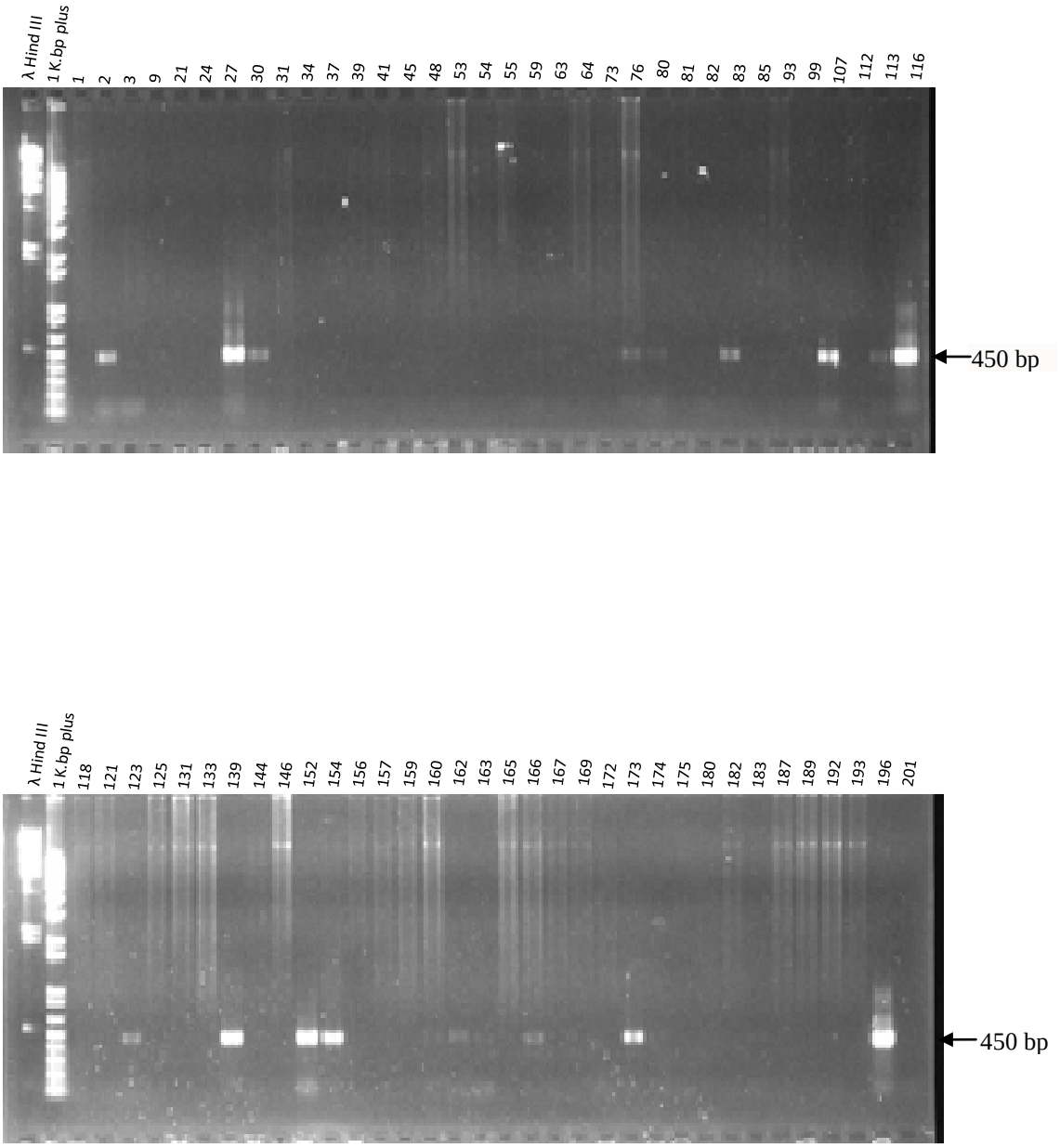
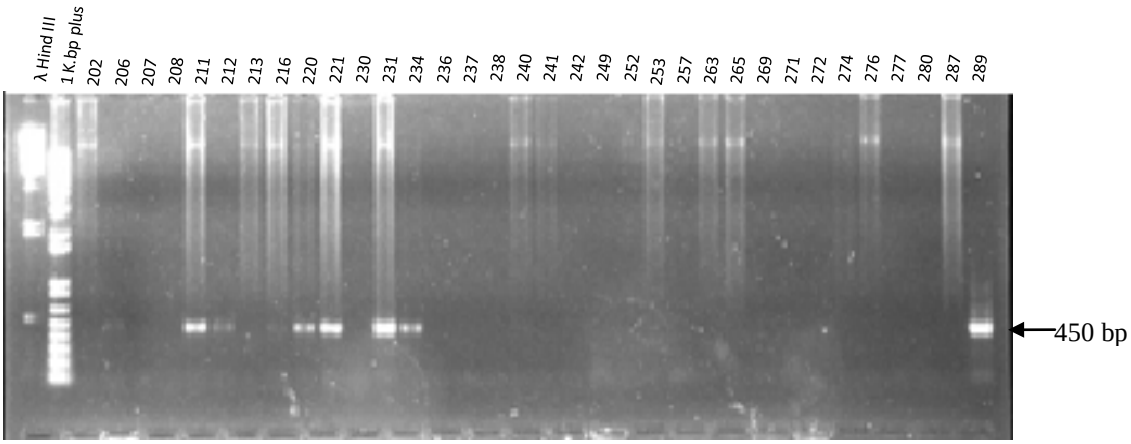


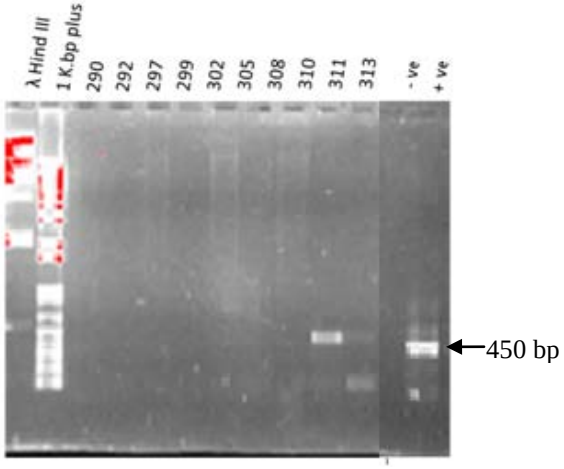
Table 6. Ct-values of the tick DNA samples given in Real time PCR and RT-PCR assays

Sample ID	Fam-*E. ewingii*	Tet-*E. chaffeensis*	Rox-*Ehrlichia/Anaplasma* Common
24i	0	0	0
27i	0	0	27.37
48i	0	0	28.46
54i	0	0	29.88
105i	0	0	28.45
112i	0	0	0
124i	0	0	0
131a	0	0	26.66
131i	0	0	0
144i	0	0	0
152i	0	0	28.31
154i	0	0	29.28
157i	0	29.15	24.17
159i	0	0	26.52
162i	0	0	30.54
163i	0	0	25.43
166i	0	30.78	26.67
169i	0	0	27.98
173i	0	0	29.58
175i	0	0	30.83
180i	0	0	26.35
211i	0	0	28.23
217i	0	28.47	24.73
229i	0	0	28.86
230i	0	0	30.66
231i	0	0	29.91
238i	0	0	28.06
241i	0	0	24.94
245i	0	0	30.51
257i	0	0	0
262i	0	0	0
269i	0	0	27.17
284a	25.87	0	26.32
290i	0	0	29.37
302i	0	0	0
313a	0	0	26.84
313i	0	0	30.09

Fig 4. Amplified PCR products of 450 bp fragment 16S rRNA gene of *Ehrlichia /Anaplasma species* of deer blood samples resolved on 1% agarose gel.

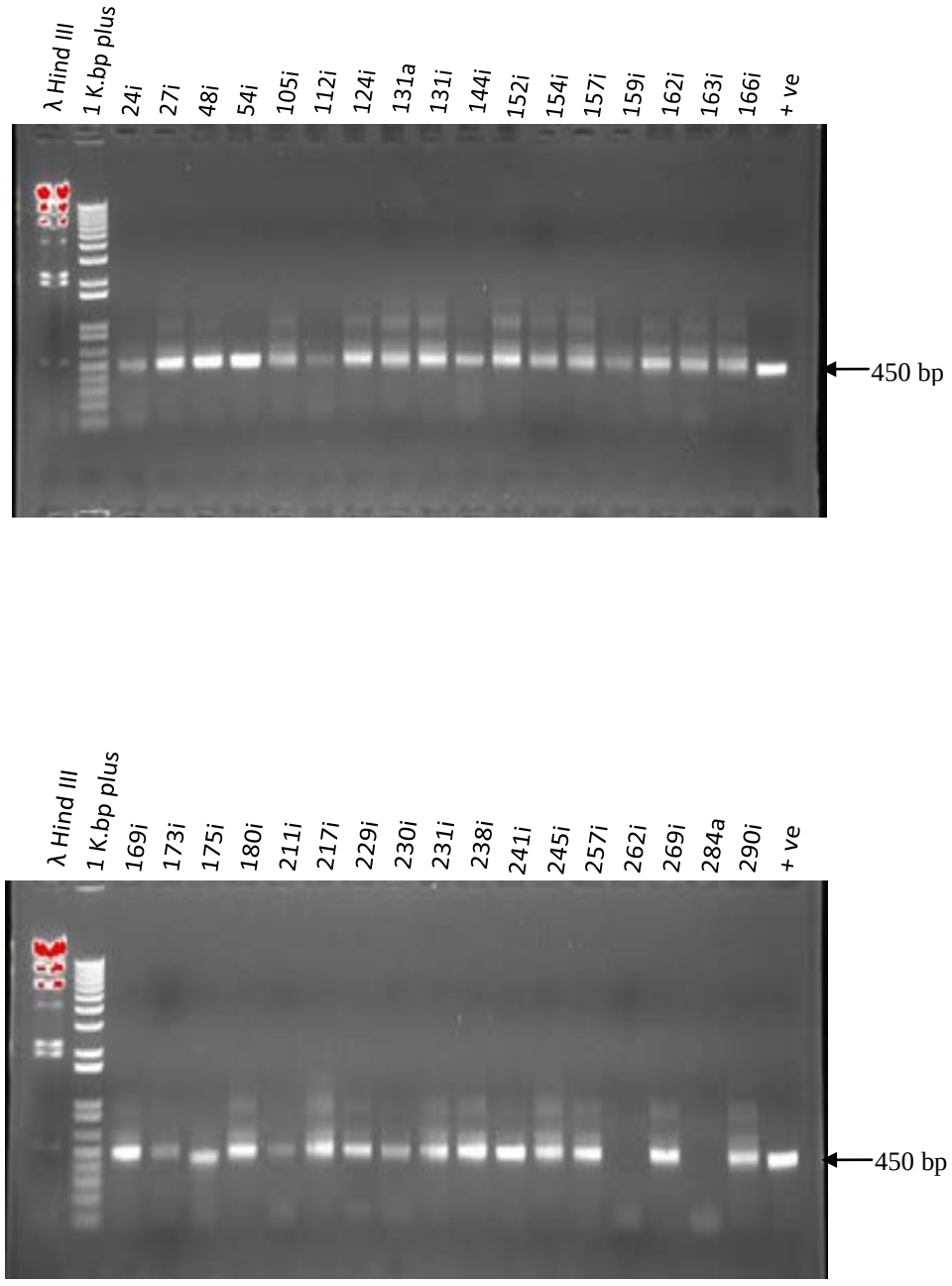


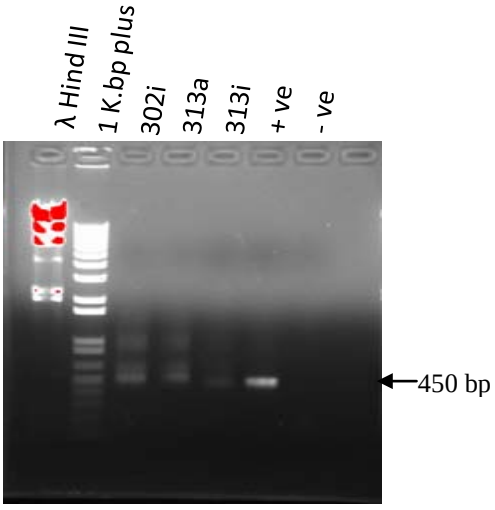




The PCR products of tick DNA samples resolved on a 1% agarose gel for identifying the presence of specific amplified fragments of 16S rRNA gene of *Ehrlichia /Anaplasma* species (450 bp). In this analysis, λ-Hind III DNA marker and 1 kb plus molecular weight markers were used for determing molecular weights and also to estimate PCR DNA concentration. The numbers represent the sample identification numbers of DNA or RNA isolated from deer blood samples.

Fig 5. Amplified PCR products of 450 bp fragment of 16S rRNA gene of *Ehrlichia /Anaplasma* species evaluated in tick derived DNA.





The PCR products of tick DNA samples resolved on a 1% agarose gel for identifying the presence of specific amplified fragments of 16S rRNA gene of *Ehrlichia /Anaplasma* species (450 bp). In this analysis, λ-Hind III DNA marker and 1 kb plus molecular weight markers were used for determing molecular weights and also to estimate PCR DNA concentration. The numbers represent the sample identification numbers of DNA isolated from tick samples.

DNA sequencing:

To establish the identity of *Ehrlichia* or *Anaplasma* species present in the samples analyzed, DNA sequencing analysis was performed on PCR products. Amplicons were purified after resolving on a 1% agarose gel and then gel isolated the predicted fragments. The gel purified DNAs were used for sequence analysis. The sequence analysis was performed for 24 amplicons derived from deer blood nucleic acids. The sequences were then evaluated by subjecting to BLAST search analysis to establish the identity of an organism present in the deer blood. These analyses identified all 24 DNAs to be nearly identical to an unnamed *Ehrlichia* species commonly found in white-tailed deer (74). The sequence alignments for only two sequences out of 24 derived from the deer blood were presented in Fig. 6 as all of them are very similar. It is regarded as the *Ehrlichia* species GA isolate No. 4 (Genebank # gb|U27104.1|ESU27104). Typically the homology for the sequences ranged from 92-99%. The second closest homology identified for these sequences is with *Anaplasma* species WTD 81 isolate (Genebank # gb|DQ007352.1|). The *Ehrlichia* species GA isolate No. 4 and *Anaplasma* species WTD 81 isolate are nearly identical for the entire sequence except for two nucleotides difference (which may represent sequence errors) (Fig. 7).

Twenty PCR amplicons derived from tick pools were also sequenced to establish the identity of bacterial organisms present in them. The BLAST search analysis of the tick sequences identified one endosymbiont of *Rickettsia* species (one tick pool), one *Alcaligenes faecalis* strain (three tick pools), five different *Pseudomonas* species (9 tick pools) and five different uncultured bacteria organisms (7 tick pools) (Table.7). The tick DNA sequences analzed through BLAST search are presented in Fig. 8. The sequence analysis of the sequences did not identify any positives for any known *Ehrlichia* or *Anaplasma* species. Careful analysis of the fluorescence

peaks of the sequence data (Fig. 9) suggested the presence of multiple overlapping peaks, which may indicate that the amplicons may have derived from several bacterial organisms (discussed in detail in the 'Discussion' section). The sequences homology's for the BLAST hits are mostly ranged between 92-99%, matches the sequences analyzed is also similar to these found for deer blood samples. The sequence data generated for tick DNAs were further assessed to identify the homology to *Ehrlichia/ Anaplasma* species common TaqMan probes. Most of the sequences had homology to 21 of 22 bases.

Fig 6. The two sequences are shown as examples to represent all the sequences derived from white-tailed deer blood samples and their identified organisms when searched in NCBI Genebank.

Sample 1

CCGCAAGCCACAACAACAACGAGCACTCTAAGCTCGCTGTGCATACTGCCTACACGACAGAAAGAGATGATAACG
AACCCGTAGGCCATTCCTCACTCACGCGGCATAGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTG
CCTCCCGTAGGAGTCTGGACCGTATTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGACCAGCTATAGATCACTGC
CTTGGTAGGCCTTTACCCTACCAACTAGCTAATCTAACATAGGCTCATCTAGTAGCGATAAATCTTTCCCCCGCAGG
GCTTATACAGTATTACCCACCATTTCTAGTGGCTATCCCTTACTACTAGGCAGATGCCTATGCATNACTCACCCGTCT
GCCACTAACCATCCCCGTAGCAAGCTACAGAGATAATTCGTACGAC

>gb|U27104.1|ESU27104 Ehrlichia sp. GA isolate No. 4, 16S rRNA gene, partial sequence
Length=1163 Score = 608 bits (329), Expect = 8e-171
Identities = 348/357 (98%), Gaps = 5/357 (1%)
Strand=Plus/Minus

```
Query  76   AACCCGT-AGGCCATTCCTCACTCACGCGGCATAGCTGGATCAGGCTTGCGCCCATTGTC  134
            ||||| | ||||| |||||||||||||||||| |||||||||||||||||||||||||
Sbjct  363  AACCC-TAAGGCC-TTCCTCACTCACGCGGCATARCTGGATCAGGCTTGCGCCCATTGTC  306

Query  135  CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTATTCTCAGTTCCAGTGTGG  194
            |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  305  CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTA-TCTCAGTTCCAGTGTGG  247

Query  195  CTGATCATCCTCTCAGACCAGCTATAGATCACTGCCTTGGTAGGCCTTTACCCTACCAAC  254
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  246  CTGATCATCCTCTCAGACCAGCTATAGATCACTGCCTTGGTAGGCCTTTACCCTACCAAC  187

Query  255  TAGCTAATCTAACATAGGCTCATCTAGTAGCGATAAATCTTTCCCCCGCAGGGCTTATAC  314
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  186  TAGCTAATCTAACATAGGCTCATCTAGTAGCGATAAATCTTTCCCCCGCAGGGCTTATAC  127

Query  315  AGTATTACCCACCATTTCTAGTGGCTATCCCTTACTACTAGGCAGATGCCTATGCATNAC  374
            |||||||||||||||||||||||||||||||||||||||||||||| ||||||||| ||
Sbjct  126  AGTATTACCCACCATTTCTAGTGGCTATCCCTTACTACTAGGCAGATTCCTATGCATTAC  67

Query  375  TCACCCGTCTGCCACTAACCAT-CCCCGTAGCAAGCTACAGAGATAATTCGTACGAC  430
            |||||||||||||||||||||| |||||||||||||||||||||||||||||| ||||
Sbjct  66   TCACCCGTCTGCCACTAACCATNCCCCGTAGCAAGCTACAGAGATAATTCGTTCGAC  10
```

>gb|DQ007352.1| Anaplasma sp. WTD 81 16S ribosomal RNA gene, partial sequence
Length=406

Score = 604 bits (327), Expect = 1e-169
Identities = 340/346 (99%), Gaps = 4/346 (1%)
Strand=Plus/Minus

```
Query  76   AACCCGT-AGGCCATTCCTCACTCACGCGGCATAGCTGGATCAGGCTTGCGCCCATTGTC  134
            ||||| | ||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  344  AACCC-TAAGGCC-TTCCTCACTCACGCGGCATAGCTGGATCAGGCTTGCGCCCATTGTC  287

Query  135  CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTATTCTCAGTTCCAGTGTGG  194
            |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  286  CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTA-TCTCAGTTCCAGTGTGG  228

Query  195  CTGATCATCCTCTCAGACCAGCTATAGATCACTGCCTTGGTAGGCCTTTACCCTACCAAC  254
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  227  CTGATCATCCTCTCAGACCAGCTATAGATCACTGCCTTGGTAGGCCTTTACCCTACCAAC  168

Query  255  TAGCTAATCTAACATAGGCTCATCTAGTAGCGATAAATCTTTCCCCCGCAGGGCTTATAC  314
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  167  TAGCTAATCTAACATAGGCTCATCTAGTAGCGATAAATCTTTCCCCCGCAGGGCTTATAC  108

Query  315  AGTATTACCCACCATTTCTAGTGGCTATCCCTTACTACTAGGCAGATGCCTATGCATNAC  374
            |||||||||||||||||||||||||||||||||||||||||| ||||||||| ||
Sbjct  107  AGTATTACCCACCATTTCTAGTGGCTATCCCTTACTACTAGGCAGATTCCTATGCATTAC  48

Query  375  TCACCCGTCTGCCACTAACCATCCCCGTAGCAAGCTACAGAGATAA  420
            |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  47   TCACCCGTCTGCCACTAACCATCCCCGTAGCAAGCTACAGAGATAA  2
```

Sample 2

CCGCAAGCCACAACAACAACGAGCACTCTAAGCTCGCTGTGCATACTGCCTACACGACAGAAAGAGATGATAACG
AACCCGTAGGCCATTCCTCACTCACGCGGCATAGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTG
CCTCCCGTAGGAGTCTGGACCGTATTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGACCAGCTATAGATCACTGC
CTTGGTAGGCCTTTACCCTACCAACTAGCTAATCTAACATAGGCTCATCTAGTAGCGATAAATCTTTCCCCCGCAGG
GCTTATACAGTATTACCCACCATTTCTAGTGGCTATCCCTTACTACTAGGCAGATGCCTATGCATNACTCACCCGTCT
GCCACTAACCATCCCCGTAGCAAGCTACAGAGATAATTCGTACGAC

>gb|U27104.1|ESU27104 Ehrlichia sp. GA isolate No. 4, 16S rRNA gene, partial sequence
Length=1163 Score = 608 bits (329), Expect = 8e-171
Identities = 348/357 (98%), Gaps = 5/357 (1%)
Strand=Plus/Minus

```
Query  76   AACCCGT-AGGCCATTCCTCACTCACGCGGCATAGCTGGATCAGGCTTGCGCCCATTGTC  134
            ||||| | ||||| |||||||||||||||||| |||||||||||||||||||||||||
Sbjct  363  AACCC-TAAGGCC-TTCCTCACTCACGCGGCATARCTGGATCAGGCTTGCGCCCATTGTC  306

Query  135  CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTATTCTCAGTTCCAGTGTGG  194
            |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  305  CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTA-TCTCAGTTCCAGTGTGG  247

Query  195  CTGATCATCCTCTCAGACCAGCTATAGATCACTGCCTTGGTAGGCCTTTACCCTACCAAC  254
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  246  CTGATCATCCTCTCAGACCAGCTATAGATCACTGCCTTGGTAGGCCTTTACCCTACCAAC  187

Query  255  TAGCTAATCTAACATAGGCTCATCTAGTAGCGATAAATCTTTCCCCCGCAGGGCTTATAC  314
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  186  TAGCTAATCTAACATAGGCTCATCTAGTAGCGATAAATCTTTCCCCCGCAGGGCTTATAC  127

Query  315  AGTATTACCCACCATTTCTAGTGGCTATCCCTTACTACTAGGCAGATGCCTATGCATNAC  374
            |||||||||||||||||||||||||||||||||||||||||| ||||||||| ||
Sbjct  126  AGTATTACCCACCATTTCTAGTGGCTATCCCTTACTACTAGGCAGATTCCTATGCATTAC  67

Query  375  TCACCCGTCTGCCACTAACCAT-CCCCGTAGCAAGCTACAGAGATAATTCGTACGAC  430
            |||||||||||||||||||||| |||||||||||||||||||||||||| ||||
Sbjct  66   TCACCCGTCTGCCACTAACCATNCCCCGTAGCAAGCTACAGAGATAATTCGTTCGAC  10
```

> gb|DQ007352.1| Anaplasma sp. WTD 81 16S ribosomal RNA gene, partial sequence

```
Length=406
Score =  604 bits (327),  Expect = 1e-169
 Identities = 340/346 (99%), Gaps = 4/346 (1%)
 Strand=Plus/Minus

Query  76   AACCCGT-AGGCCATTCCTCACTCACGCGGCATAGCTGGATCAGGCTTGCGCCCATTGTC  134
            ||||| | ||||| |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  344  AACCC-TAAGGCC-TTCCTCACTCACGCGGCATAGCTGGATCAGGCTTGCGCCCATTGTC  287

Query  135  CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTATTCTCAGTTCCAGTGTGG  194
            |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  286  CAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTA-TCTCAGTTCCAGTGTGG  228

Query  195  CTGATCATCCTCTCAGACCAGCTATAGATCACTGCCTTGGTAGGCCTTTACCCTACCAAC  254
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  227  CTGATCATCCTCTCAGACCAGCTATAGATCACTGCCTTGGTAGGCCTTTACCCTACCAAC  168

Query  255  TAGCTAATCTAACATAGGCTCATCTAGTAGCGATAAATCTTTCCCCCGCAGGGCTTATAC  314
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  167  TAGCTAATCTAACATAGGCTCATCTAGTAGCGATAAATCTTTCCCCCGCAGGGCTTATAC  108

Query  315  AGTATTACCCACCATTTCTAGTGGCTATCCCTTACTACTAGGCAGATGCCTATGCATNAC  374
            |||||||||||||||||||||||||||||||||||||||||| ||||||||| ||
Sbjct  107  AGTATTACCCACCATTTCTAGTGGCTATCCCTTACTACTAGGCAGATTCCTATGCATTAC  48

Query  375  TCACCCGTCTGCCACTAACCATCCCCGTAGCAAGCTACAGAGATAA  420
            |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  47   TCACCCGTCTGCCACTAACCATCCCCGTAGCAAGCTACAGAGATAA  2
```

Fig 7. The sequences of 16S rRNA gene segment of Anaplasma sp. WTD 81 and Ehrlichia sp. GA isolate No. 4 in NCBI Genebank and also alignment of these sequences.

Anaplasma sp. WTD 81 16S rRNA gene, partial sequence

```
   1 tttatctctg tagcttgcta cggggatggt tagtggcaga cgggtgagta atgcatagga
  61 atctgcctag tagtaaggga tagccactag aaatggtggg taatactgta taagccctgc
 121 gggggaaaga tttatcgcta ctagatgagc ctatgttaga ttagctagtt ggtagggtaa
 181 aggcctacca aggcagtgat ctatagctgg tctgagagga tgatcagcca cactggaact
 241 gagatacggt ccagactcct acgggaggca gcagtgggga atattggaca atgggcgcaa
 301 gcctgatcca gctatgccgc gtgagtgagg aaggccttag ggttgtaaaa ctctttcagt
 361 ggggaagata atgacggtac ccacagaaga agtcccggca aactca
```

Ehrlichia sp. GA isolate No. 4 16S rRNA gene, partial sequence

```
   1 cacatgcaag tcgaacgaat tatctctgta gcttgctacg gggnatggtt agtggcagac
  61 gggtgagtaa tgcataggaa tctgcctagt agtaagggat agccactaga aatggtgggt
 121 aatactgtat aagccctgcg ggggaaagat ttatcgctac tagatgagcc tatgttagat
 181 tagctagttg gtagggtaaa ggcctaccaa ggcagtgatc tatagctggt ctgagaggat
 241 gatcagccac actggaactg agatacggtc cagactccta cgggaggcag cagtggggaa
 301 tattggacaa tgggcgcaag cctgatccag ytatgccgcg tgagtgagga aggccttagg
 361 gttgtaaaac tctttcagtg gggaagataa tgacggtacc cacagaagaa gtcccggcaa
 421 actccgtgcc agcagccgcg gtaatacgga gggggcaagc gttgttcgga attattgggc
 481 gtaaagggca tgtaggcggt tcggtaagtt aaaggtgaaa tgccagggct taaccctgga
 541 gctgctttta atactgccag actagagacc gggagaggat agcggaattc ctagtgtaga
 601 ggtgaaattc gtagatatta ggaggaacac cagtggcgaa ggcggctatc tggtccggta
 661 ctgacgctga ggtgcgaaag cgtggggagc aaacaggatt agataccctg gtagtccacg
 721 ctgtaaacga tgagtgctga atgtgggggt gttttacctc cgtgttgtag ctaacgcgtt
 781 aagcactccg cctggggact acggtcgcaa gactaaaact caaaggaatt gacggggacc
 841 cgcacaagcg gtggagcatg tggtttaatt cgatgcaacg cgaagaacct taccacttct
 901 tgacatggag attagatcct tcttaacgga agggcgcagt tcggctggat ctcgcacagg
 961 tgctgcatgg ctgtcgtcag ctcgtgtcgt gagatgttgg gttaagtccc gcaacgagcg
1021 taaccctcat ccttagttgc cagcgggtta agccgggcac tttaaggaga ctgccagtgg
1081 taaactggag gaaggtgggg atgatgtcaa gtcagcacgg cccttatggg gtgggckaca
1141 cacgtgctac aatggtgacd aca
```

Anaplasma sp. WTD 81 v/s Ehrlichia sp. GA isolate No. 4

```
lcl|15729
Length=1163

 Score =  737 bits (399),  Expect = 0.0
 Identities = 403/405 (99%), Gaps = 1/405 (0%)
 Strand=Plus/Plus

Query  2    TTATCTCTGTAGCTTGCTACGGGG-ATGGTTAGTGGCAGACGGGTGAGTAATGCATAGGA  60
            |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  20   TTATCTCTGTAGCTTGCTACGGGGNATGGTTAGTGGCAGACGGGTGAGTAATGCATAGGA  79

Query  61   ATCTGCCTAGTAGTAAGGGATAGCCACTAGAAATGGTGGGTAATACTGTATAAGCCCTGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  80   ATCTGCCTAGTAGTAAGGGATAGCCACTAGAAATGGTGGGTAATACTGTATAAGCCCTGC  139

Query  121  GGGGGAAAGATTTATCGCTACTAGATGAGCCTATGTTAGATTAGCTAGTTGGTAGGGTAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  140  GGGGGAAAGATTTATCGCTACTAGATGAGCCTATGTTAGATTAGCTAGTTGGTAGGGTAA  199

Query  181  AGGCCTACCAAGGCAGTGATCTATAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  200  AGGCCTACCAAGGCAGTGATCTATAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACT  259

Query  241  GAGATACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  260  GAGATACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAA  319

Query  301  GCCTGATCCAGCTATGCCGCGTGAGTGAGGAAGGCCTTAGGGTTGTAAAACTCTTTCAGT  360
            |||||||||||  ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  320  GCCTGATCCAGYTATGCCGCGTGAGTGAGGAAGGCCTTAGGGTTGTAAAACTCTTTCAGT  379

Query  361  GGGGAAGATAATGACGGTACCCACAGAAGAAGTCCCGGCAAACTC  405
            |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  380  GGGGAAGATAATGACGGTACCCACAGAAGAAGTCCCGGCAAACTC  424
```

Fig 8. The sequences derived from 20 tick DNA samples and the organisms identified by them when searched in NCBI Genebank.

24 i

TACAGACGTTTATACTCAGCTGCTTATGCTGTCGCGTAACGTCAAAACATGTCAAGGTATTACGCGTAACTGCCCTT
CCTCCCAACTTAAAGTGCTTTACAATCCGAAGAGCCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCA
TTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCT
CAGACCAGTTACGGATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATA
GCGCAAGGCCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCCTTTCCAAACGTNATCCC
CCACTACCAGGCAGATCCCTATGTATTACTCACCCGTCCGCCGCTCACTAGAGGAGCAAGATCTTCTCATCCGTGCG
ACTTGCATGTGTAAGGCAAGAGAACGAGACCCCCCTCGCAAAAAAACACAAC

> gb|DQ158108.1| Uncultured bacterium clone 516 16S ribosomal RNA gene, partial sequence
Length=1512 Score = 719 bits (389), Expect = 0.0
Identities = 436/458 (96%), Gaps = 8/458 (1%)
Strand=Plus/Minus

```
Query  21   TGCTTATGCTGTCGCGTAACGTCAAAACATGTCAAGGTATTACGCGTAACTGCCCTTCCT  80
            ||||||| |||||| |||||||||||||| | ||||||||| ||| | ||||||||||||
Sbjct  494  TGCTTATTCTGTCG-GTAACGTCAAAACA-G-CAAGGTATT-CGC-TTACTGCCCTTCCT  440

Query  81   CCCAACTTAAAGTGCTTTACAATCCGAAGAGCCTTCTTCACACACGCGGCATGGCTGGAT  140
            ||||||||||||||||||||||||||||| |||||||||||||||||||||||||||||
Sbjct  439  CCCAACTTAAAGTGCTTTACAATCCGAAGA-CCTTCTTCACACACGCGGCATGGCTGGAT  381

Query  141  CAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGT  200
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  380  CAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGT  321

Query  201  GTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTG  260
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  320  GTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTG  261

Query  261  AGCCATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGA  320
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  260  AGCCATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGA  201

Query  321  AGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCCTTTCCAAACGTNAT  380
            |||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||  |
Sbjct  200  AGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCCTTTCGAAACGTTGT  141

Query  381  CCCCCACTACCAGGCAGATCCCTATGTATTACTCACCCGTCCGCCGCTCACTAGAGGAGC  440
            |||||||||||||||||| |||| | |||||||||||||||||| | | || ||||
Sbjct  140  CCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTGAATCGAAGAGC  81

Query  441  AAGATCTTCTCATCCG-TGCGACTTGCATGTGTAAGGC  477
            ||| |||||||||||| | |||||||||||||| ||||
Sbjct  80   AAGCTCTTCTCATCCGCT-CGACTTGCATGTGTTAGGC  44
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  1   TAACACATGCAAGTCGAACGGA  22
           |||||||||||||||| ||||
Sbjct  47  TAACACATGCAAGTCGAGCGGA  68
```

27 i

ACGATGCTCTTTACTTCTCATATGTGCGCAGTGCGCGCGCGATGCTCGGGCGTGTGTACATGNGTGATGAATGCAT
AACTAGCACGTACGCGCGCACACTCGCTCGCACGTGTGAGTGAGGTACGCGGCGCGCACTACACGCTACATCGGA
ACATACGTATGTTATCGCTCTACATTACGCTGCGCACTGATCGCGACTATACAGCGACTGTAGAGACGCCGCGGGG
CGCACTCCTCTCCGCGCACCTTCGCACGCTATACTAAACGCACTGGAGGCCCCTAATTGCTAAACGAATTAGGCTTA
GGTTGGGGTGGGGGGCTACAACCGCCCCGCCAAAGGCCGACGATCGTTTTCCGTGGTATGAGAGGATGATCAGC
CACACTGGGACTGAGACACGGCCCAGACTCCTAGGGGAGGCAGCAATGGGGAATATTGGACAATGGGTGGAAG
CCTGATCCAGCAATTCCTGTGTGTGTGAAAGAACGGTGTTCGGAGACTTCATATACCGCTAGCGTGTGGAGAGAT
GCGAGGGAGGGAACTGCGTACGCGCTGGTGAGCGTTTATGATCAGCCGCCGCTTCATACCATACTCANCGCCTNT
GGTGACCTGGCGCCTNTTAAGTGAGCGCACTTTGCTCACACCGCGTAAGAACACGCACACTACGCGCAGCNGTGC
GCTNGACACTGGCGGTGTAGCCCGGGCNGAGCGCTCACGTGAGAAGATGCATAA

> gb|HM069816.1| Uncultured bacterium clone Bacteria_Clone_349 16S ribosomal RNA
gene, partial sequence
Length=399

Score = 200 bits (108), Expect = 1e-47
Identities = 143/159 (90%), Gaps = 6/159 (3%)
Strand=Plus/Plus

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Query  337  AAGGCCGACGATCGTTTTCCGTGGTATGAGAGGATGATCAGCCACACTGGGACTGAGACA  396
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Sbjct  240  AAGG-CGACGATCG-TTAGC-TGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACA  296

Query  397  CGGCCCAGACTCCTAGGGGAGGCAGCAATGGGGAATATTGGACAATGGGTGGAAGCCTGA  456
            |||||||||||||| |||||||||| |||||||||||||||||||| | ||||||||
Sbjct  297  CGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGA  356

Query  457  TCCAGCAATTCCTGTGTGTGTGAAAGAACGGTGTTCGGA  495
            ||||||||| || | ||||||||| ||| ||| ||||||
Sbjct  357  TCCAGCAATGCC-GCGTGTGTGAA-GAA-GGTCTTCGGA  392
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  1   TAACACATGCAAGTCGAACGG  21
           |||||||||||||||| ||||
Sbjct  42  TAACACATGCAAGTCGCACGG  62
```

48 i

GTGCTTATGCTGTCGGTAACGTCAAAATTGCAGAGTATGTAATGCTACAACCCTTCCTCCCAACTTAAAGTGCTTTA
CAATCCGAAGACCTTCTTCACACACGACGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCT
GCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCG
CCTTGGTGAGCCATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCC
TGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCCCCCACTACCAGGCAGATTCCTAG
GCATTACTCACCCGTCCGCCGCTCTCAAGAGAAGCAAGCTTCTCTCTACCGTTCGACTTGCATGTGTAAGGCAAAG
AGTCCTG

>gb|HM561497.1| Uncultured Pseudomonas sp. clone Dn12 16S ribosomal RNA gene, partial sequence
Length=652

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 Score =  804 bits (435),  Expect = 0.0
 Identities = 449/455 (99%), Gaps = 3/455 (0%)
 Strand=Plus/Minus

Query  1    GTGCTTATGCTGTCGGTAACGTCAAAATTGCAGAGTATGTAATGCTACAACCCTTCCTCC  60
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Sbjct  490  GTGCTTATTCTGTCGGTAACGTCAAAATTGCAGAGTAT-TAAT-CTACAACCCTTCCTCC  433

Query  61   CAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGACGGCATGGCTGGATCA  120
            |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  432  CAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACG-CGGCATGGCTGGATCA  374

Query  121  GGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  373  GGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGT  314

Query  181  CTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  313  CTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAG  254

Query  241  CCATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  253  CCATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAG  194

Query  301  GTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  193  GTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCC  134

Query  361  CCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTCTCAAGAGAAGCAA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  133  CCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTCTCAAGAGAAGCAA  74

Query  421  GCTTCTCTCTACCGTTCGACTTGCATGTGTAAGGC  455
            ||||||||||||| ||||||||||||||| ||||
Sbjct  73   GCTTCTCTCTACCGCTCGACTTGCATGTGTTAGGC  39
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  1   TAACACATGCAAGTCGA  17
           |||||||||||||||||
Sbjct  42  TAACACATGCAAGTCGA  58
```

54 i

TTATTCTGTCGGTAACGTCAAAACACTAACGTATTAGGTTAATGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCG
AAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCG
TAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTG
AGCCATTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTCTC
CCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCCCCCACTACCAGGCAGATTCCTAGGTATTACTC
ACCCGTCCGCCGCTCTCAAGAGGTGCAAGCACCTCTCTACCGTTCGACTTGCATGTGTAAGGACAGAGANNNN

>gb|HM332859.1| Uncultured bacterium clone ncd1064f04c1 16S ribosomal RNA gene, partial sequence

```
Length=1358   Score =  815 bits (441),  Expect = 0.0
 Identities = 445/447 (99%), Gaps = 0/447 (0%)
 Strand=Plus/Minus

Query  1    TTATTCTGTCGGTAACGTCAAAACACTAACGTATTAGGTTAATGCCCTTCCTCCCAACTT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  466  TTATTCTGTCGGTAACGTCAAAACACTAACGTATTAGGTTAATGCCCTTCCTCCCAACTT  407

Query  61   AAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  406  AAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTC  347

Query  121  GCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  346  GCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTT  287

Query  181  CCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTAC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  286  CCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTAC  227

Query  241  CTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  226  CTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCT  167

Query  301  GCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCCCCCACTA  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  166  GCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCCCCCACTA  107

Query  361  CCAGGCAGATTCCTAGGTATTACTCACCCGTCCGCCGCTCTCAAGAGGTGCAAGCACCTC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  106  CCAGGCAGATTCCTAGGTATTACTCACCCGTCCGCCGCTCTCAAGAGGTGCAAGCACCTC  47

Query  421  TCTACCGTTCGACTTGCATGTGTAAGG  447
            ||||||| ||||||||||||||| |||
Sbjct  46   TCTACCGCTCGACTTGCATGTGTTAGG  20
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  1   TAACACATGCAAGTCGA  17
           |||||||||||||||||
Sbjct  22  TAACACATGCAAGTCGA  38
```

105 i

AGTCCTATGATTGCTGGTCGAGTAACGTGCAAAACAGTCAAATGATGTAGTGTAACTGCCCTTCCTCCCACGCTTA
AAGTGCTTTACAATCCTAAGTAGCCTTCTTCACACACGCGGCATGGCTGGATGCAGGGGTTTCCCCCATTGTCCAAT
ATTCCGCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGT
TACTCATCGCGGTCTTGGTGAGCCATTACCTCACCAACTAACTAATCCGACCTAGGCTCATCTAATAGCGAAAGGCT
CCAAAGAGTCCCCTCCTTTCTCCCGTAGGACGTATGCGGTATTAGCTTACCTTTCGGCAAGTTATCCCCCACTACTA
GGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTCGTCAGCAAAGAAGCAAGTCTTCTTCCTGTTACCGTTCG
ACTTGCATGTGTAAAGCTAAG

> gb|AY017062.1|AY017062S1 Pseudomonas sp. CL-2 16S ribosomal RNA gene, 5'-partial sequence
Length=816

Score = 723 bits (391), Expect = 0.0
Identities = 440/461 (96%), Gaps = 13/461 (2%)
Strand=Plus/Minus

```
Query  17   GTCGAGTAACGTGCAAAACAGTCAAATGATGTAGTGTAACTGCCCTTCCTCCCACGCTTA  76
            |||| ||||||| ||||||||||||||| || |||| ||||||| ||||||||||  ||||
Sbjct  493  GTCG-GTAACGT-CAAAACAGTCAAAT-AT-TAGT-TAACTGCTCTTCCTCCCA-ACTTA  440

Query  77   AAGTGCTTTACAATCCTAAGTAGCCTTCTTCACACACGCGGCATGGCTGGATGCAGGGGT  136
            |||||||||||||||||| | |||||||||||||||||||||||||||| || ||||
Sbjct  439  AAGTGCTTTACAATCCTAAG-A-CCTTCTTCACACACGCGGCATGGCTGGAT-CA-GGGT  384

Query  137  TTCCCCCATTGTCCAATATTCCGCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCA  196
            |||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  383  TTCCCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCA  324

Query  197  GTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACTCATCGCGGTCTTGGTGAGCCAT  256
            |||||||||||||||||||||||||||||||||||  ||||||||||||||||||||||
Sbjct  323  GTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGCGGTCTTGGTGAGCCAT  264

Query  257  TACCTCACCAACTAACTAATCCGACCTAGGCTCATCTAATAGCGAAAGGCTCCAAAGAGT  316
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  263  TACCTCACCAACTAACTAATCCGACCTAGGCTCATCTAATAGCGAAAGGCTCCGAAGAGT  204

Query  317  CCCCTCCTTTCTCCCGTAGGACGTATGCGGTATTAGCTTACCTTTCGGCAAGTTATCCCC  376
            |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct  203  CCCCTCCTTTCTCCCGTAGGACGTATGCGGTAT-AGCTTACCTTTCGGCAAGTTATCCCC  145

Query  377  CACTACTAGGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTCGTCAGCAAAGAAGC  436
            |||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  144  CACTACTAGG-CAGATTCCTAGGCATTACTCACCCGTCCGCCGCTCGTCAGCAAAGAAGC  86

Query  437  AAGTCTTCTTCCTGTTACCGTTCGACTTGCATGTGTAAAGC  477
            ||| |||||||||||||| ||||||||||||||| ||||
Sbjct  85   AAG-CTTCTTCCTGTTACCGCTCGACTTGCATGTGTTAAGC  46
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  49  TAACACATGCAAGTCGAGCGG  69
           |||||||||||||||| |||
Sbjct  1   TAACACATGCAAGTCGAACGG  21
```

112 i

TCAGTGCTTATTGCTGTCGGTAACGTCAAAATCTAGCAAAGTATTAGTGTAACATGCCCTTCCTCCCATACTCAAAG
TGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCC
ACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGA
TCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAG
GTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCAAACGTTATCCCCCACTACCAGGCAGAT
TCCTAGGCATTACTCACCCGTCCGCCGCTCTCAAGAGAAGCAAGCTCCTCTCTACCGTTCGACTTGCATGTG

> gb|EF192826.1| Uncultured bacterium clone IM4_H04 16S ribosomal RNA gene, partial sequence
Length=929
Score = 769 bits (416), Expect = 0.0
Identities = 441/452 (98%), Gaps = 6/452 (1%)
Strand=Plus/Minus

```
Query  4    GTGCTTATTGCTGTCGGTAACGTCAAAATCTAGCAAAGTATTAGTGTAACATGCCCTTCC  63
            ||||||||| |||||||||||||||||| | |||||||||||| ||| || |||||||||
Sbjct  450  GTGCTTATT-CTGTCGGTAACGTCAAAA-C-AGCAAAGTATTAATGT-AC-TGCCCTTCC  396

Query  64   TCCCATACTCAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGA  123
            ||||| ||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  395  TCCCA-ACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGA  337

Query  124  TCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCG  183
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  336  TCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCG  277

Query  184  TGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGT  243
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  276  TGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGT  217

Query  244  GAGCCATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCG  303
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  216  GAGCCATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCG  157

Query  304  AAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCAAACGTTA  363
            |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  156  AAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTA  97

Query  364  TCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTCTCAAGAGAAG  423
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  96   TCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTCTCAAGAGAAG  37

Query  424  CAAGCTCCTCTCTACCGTTCGACTTGCATGTG  455
            |||||| |||||||||| ||||||||||||||
Sbjct  36   CAAGCTTCTCTCTACCGCTCGACTTGCATGTG  5
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  3   ACACATGCAAGTCGA  17
           |||||||||||||||
Sbjct  4   ACACATGCAAGTCGA  18
```

124 i

GTGACTTAATTGCTGGTCCGAGTAACGTGCAAAACAGTACACGATGATGTAGTGTCA
ACTGGCCCTTCCTCCCAACTTGAAAGTGCTTTACAATCCTAAGACCTTCTTCACACAC
GCGGCATTGGCTGGATCAGGCTTTCCCCCATTGTCCAATATTCCCCACTGCTGCCTCC
CGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAG
TTACGGATCGTGGCCTTGGTGAGCCATTACCTCACCAACTAACTAATCCGACCTAGG
CTCATCTAATAGCGACAAGGCCCGAAGGTCCCCTCCTTTCTCCCGTAGGACATATGC
TGCGTATATATGACGCACCCTTTCCGCGACAACGTTTTTTCCCCCCCCCACNTAACTT
AGGGGGCAGGCAAATTATTCCCTAAGTGAGGCTAGNTATATCTATCCCATCCCCCCT
GNCTGCNCNGCCCCGGNCNTNCGTCTATATAGTAAGAAAAAGAATAAATATCAATA
TACNTCTCTCTCTTCCNTCGCTNTNCTCGCACGCTTGGCCCGCACGANTNGACTTNAT
AGATGGCGTACTATAATAGAACCAGA

> gb|FJ950669.1| Pseudomonas sp. d130 16S ribosomal RNA gene, partial sequence

```
Length=1459

 Score =  496 bits (268),  Expect = 9e-137
 Identities = 323/346 (94%), Gaps = 17/346 (4%)
 Strand=Plus/Minus

Query  1    GTGACTTAATTGCTGGTCCGAGTAACGTGCAAAACAGTACACGATGATGTAGTGTCAACT  60
            ||| ||||||| || || || ||||||| ||||||||| ||  || || |||| | ||||
Sbjct  469  GTG-CTTAATT-CT-GTTCG-GTAACGT-CAAAACAGT-CA-AAT-AT-TAGT-T-AACT  421

Query  61   GGCCCTTCCTCCCAACTTGAAAGTGCTTTACAATCCTAAGACCTTCTTCACACACGCGGC  120
             ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  420  -GCCCTTCCTCCCAACTT-AAAGTGCTTTACAATCCTAAGACCTTCTTCACACACGCGGC  363

Query  121  ATTGGCTGGATCAGGCTTTCCCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGA  180
            | |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  362  A-TGGCTGGATCAGGGTTTCCCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGA  304

Query  181  GTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  303  GTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCG  244

Query  241  TGGCCTTGGTGAGCCATTACCTCACCAACTAACTAATCCGACCTAGGCTCATCTAATAGC  300
             || ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  243  CGGTCTTGGTGAGCCATTACCTCACCAACTAACTAATCCGACCTAGGCTCATCTAATAGC  184

Query  301  GACAAGGC-CCGAAG-GTCCCCTCCTTTCTCCCGTAGGACATATGC  344
            || ||||| |||||| ||||||||||||||||||||||| |||||
Sbjct  183  GA-AAGGCTCCGAAGAGTCCCCTCCTTTCTCCCGTAGGACGTATGC  139
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  4    CACATGC  10
            |||||||
Sbjct  922  CACATGC  916
```

131A

AGGCTATTATTGCTGCAGATACGCTCAGCATTATCTCGGTATGTAGGGAGGATACCTTTTCTTCTCTGCCAAAAGTA
CTTTACAACCCGAAGTGGGATCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCAAAATTCCCC
ACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGA
TCGTCAGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAATAGTGAGAGGTCTCGA
ACGATAAGCCCCTTTCCCGAAGTATGGCGTATGCGGTATAAGCCTCTCTTTCGAGTATTGATCCCCGGCTACTGGG
CACGTGTCGATATATAACTCACCCGTGCGCCACTCGCCGCCAAAAGATGCACTGCTCTCCTCTGCTGCGTCGCACG
CTTTCCGCACATTCGTTCACTATGTAGGGTGGAGAGCGAAC

> gb|GU181289.1| Alcaligenes faecalis strain WT10 16S ribosomal RNA gene, partial sequence
Length=1536

Score = 586 bits (317), Expect = 4e-164
Identities = 400/437 (92%), Gaps = 18/437 (4%)
Strand=Plus/Minus

```
Query  7    TTATTGCTGCAGATA-CGCTCAGCATTATCTCGGTATGTAGGGAGGATACCTTTTCTTCT  65
            ||||| ||||||||| || |||||| |||||| |||| || ||| ||||||||||||||
Sbjct  497  TTATT-CTGCAGATACCG-TCAGCAGTATCTC-GTAT-TA-GGA-GATACCTTTTCTTCT  444

Query  66   CTGCCAAAAGTACTTTACAACCCGAAGTGGGATCATCATACACGCGGGATGGCTGGATCA  125
            |||||||||||||||||||||||||| |   ||||||||||||||||||||||||||||
Sbjct  443  CTGCCAAAAGTACTTTACAACCCGAAG-GCCTTCATCATACACGCGGGATGGCTGGATCA  385

Query  126  GGGTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGT  185
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  384  GGGTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGT  325

Query  186  CTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTCAGCCTTGGTGA  245
            |||||||||||||||||||||||||||||||||||||||||||||||||  ||||||||||
Sbjct  324  CTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTT-GCCTTGGTGA  266

Query  246  GCCTTTACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAATAGTGAGAGGTCTCGA  305
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  265  GCCTTTACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAATAGTGAGAGGTCTTG-  207

Query  306  ACGATAAGCCCCTTTCCCGAAGTATGGCGTATGCGGTATAAGCCTCTCTTTCGAGTA-TT  364
             ||||   ||||||||||   ||| ||||||||||||| |||| ||||||||||| ||
Sbjct  206  -CGATCCCCCCCTTTCCCCC-GTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTT  149

Query  365  GATCCCCGGCTACTGGGCACGTGTC-GATATATAACTCACCCGTGCGCCACTCGCCGCCA  423
             |||||| |||||||||||||| || ||||||| |||||||||| ||||||||||||||
Sbjct  148  -ATCCCCCGCTACTGGGCACGT-TCCGATATATTACTCACCCGTCCGCCACTCGCCGCCA  91

Query  424  AAAGATGCACTGCTCTC  440
            | ||| |||  ||||||
Sbjct  90   AGAGA-GCAA-GCTCTC  76
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  49  ACACATGCAAGTCGAACGG  67
           ||||||||||||||||||
Sbjct  3   ACACATGCAAGTCGAACGG  21
```

144i

ATCAAGTGCTTATGCTGTCGGTAACGTCAAAATTGCAGAGTATGTAATGCTACAACCCTTCCTCCCAACTTAAAGTG
CTTTACAATCCGAAGACCTTCTTCACACACGACGGCATGGCTGGATCAGGCTTTCCCCCATTGTCCAATATTCCCCA
CTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATC
GTCCCCTTGGTGAGCCATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGT
CCCATGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCCCCCACTACCAGGCAGATTC
CTAGGCATTACTCACCCGTCCGCCGCTCTCAAGAGAAGCAAGCTTCTCTCTACCGTTCGACTTGCATGTGTAAGGCA
AAGAGTCCTGATCATA

> gb|HM561497.1| Uncultured Pseudomonas sp. clone Dn12 16S ribosomal RNA gene, partial sequence
Length=652
Score = 787 bits (426), Expect = 0.0
Identities = 446/455 (99%), Gaps = 3/455 (0%)
Strand=Plus/Minus

```
Query  6    GTGCTTATGCTGTCGGTAACGTCAAAATTGCAGAGTATGTAATGCTACAACCCTTCCTCC  65
            |||||||| |||||||||||||||||||||||| |||| ||||||||||||||||
Sbjct  490  GTGCTTATTCTGTCGGTAACGTCAAAATTGCAGAGTAT-TAAT-CTACAACCCTTCCTCC  433

Query  66   CAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGACGGCATGGCTGGATCA  125
            |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  432  CAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACG-CGGCATGGCTGGATCA  374

Query  126  GGCTTTCCCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGT  185
            ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  373  GGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGT  314

Query  186  CTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCCCCTTGGTGAG  245
            |||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  313  CTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAG  254

Query  246  CCATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAG  305
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  253  CCATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAG  194

Query  306  GTCCCATGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCC  365
            ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  193  GTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCC  134

Query  366  CCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTCTCAAGAGAAGCAA  425
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  133  CCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTCTCAAGAGAAGCAA  74

Query  426  GCTTCTCTCTACCGTTCGACTTGCATGTGTAAGGC  460
            ||||||||||||| |||||||||||||| ||||
Sbjct  73   GCTTCTCTCTACCGCTCGACTTGCATGTGTTAGGC  39
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  1   TAACACATGCAAGTCGA  17
           |||||||||||||||||
Sbjct  42  TAACACATGCAAGTCGA  58
```

152i

CCTTATTGCTGCTACGAAGTACACGTTCAAACATATCACTACATCGTTATTAGAAGTACGGATAGACCCACTCTCCC
TCCCCATAGCCTCAAAAGTGACTTTACAATCCCGTAAGAGCCTTCTATCACTACACGCGGGATGGCTGGATCAGGC
GTTGTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACT
GATCATCCTCTCAGACCAGCTACGGATCGTCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATCCGATCTAGG
CTCATCTAATAGCGCAAGGTCCGAAGGTCCCCCCCTTTCCCCCGTAGGACGTATGCGGTATTAGCCTCTCTTTCGAG
AAGTTATCCCCCACTACCGGGCACATACCTATGCATTACTCACCCGTCCGCCACTCAACTGCAAAAACACGCACCTC
TCACCCCGCCTGGCACGATTGCGAATGGTGTTAATTGTGGCTATAAAAGAAACACCAAAAAGG

> gb|GU300357.1| Uncultured Pseudomonas sp. clone PSB011.C21_E13 16S ribosomal RNA gene, partial sequence
Length=794

Score = 496 bits (268), Expect = 8e-137
Identities = 320/344 (94%), Gaps = 8/344 (2%)
Strand=Plus/Minus

```
Query  92   AAAGTGACTTTACAATCCCGTAAGAGCCTTCTATCACTACACGCGGGATGGCTGGATCAG  151
            |||||| ||||||||| ||| |||| |||||| |||| |||||||| ||||||||||||
Sbjct  389  AAAGTG-CTTTACAAT-CCG-AAGA-CCTTCT-TCAC-ACACGCGGCATGGCTGGATCAG  336

Query  152  GCGTTGTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTG  211
            || || ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  335  GC-TT-TCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTG  278

Query  212  TCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGCTACGGATCGTCGCCTTGGTGA  271
            |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Sbjct  277  TCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGA  218

Query  272  GCCATTACCCCACCAACTAGCTAATCCGATCTAGGCTCATCTAATAGCGCAAGGTCCGAA  331
            |||||||||||||||||||||||||||| |||||||||||| |||||||||||| |||||
Sbjct  217  GCCATTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAA  158

Query  332  GGTcccccccTTTCCCCCGTAGGACGTATGCGGTATTAGCCTCTCTTTCGAGAAGTTATC  391
            |||||||  ||||| |||||||||||||||||||||||| || ||||||||| ||| ||
Sbjct  157  GGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCCTTTCGAGACGTTGTC  98

Query  392  CCCCACTACCGGGCACATACCTATGCATTACTCACCCGTCCGCC  435
            |||||||||| |||| || |||| ||||||||||||||||||||
Sbjct  97   CCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCC  54
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  1   TAACACATGCAAGTCGAACGGA  22
           |||||||||||||||| ||||
Sbjct  5   TAACACATGCAAGTCGAGCGGA  26
```

157i

TGATTCACTCATCTAGAAGAGCGGCGGACGGGTGAGTAATACCTAGGAATCTGCCTGGTAGTGGGGGATAACGTT
CGGAAACGGACGCTAATACCGCATACGTTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATG
AGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCTACAATCCGTAACTGGTCTGAGAGGATG
CTACAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGG
GGAAAGCCTGATCCAGCCATGCCGCGTGTAGGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTGGGGGAGGA
AGGGCATTAACCTAATACGTTAGTGTTTACGACGTTCACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGC
CGACAGTCGAAC

> gb|GU062533.1| Pseudomonas sp. KOPRI 25416 16S ribosomal RNA gene, partial sequence
Length=1396
Score = 730 bits (395), Expect = 0.0
Identities = 423/435 (98%), Gaps = 7/435 (1%)
Strand=Plus/Plus

```
Query  18   AGAGCGGCGGACGGGTGAGTAATACCTAGGAATCTGCCTGGTAGTGGGGGATAACGTTCG  77
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  40   AGAGCGGCGGACGGGTGAGTAATACCTAGGAATCTGCCTGGTAGTGGGGGATAACGTTCG  99

Query  78   GAAACGGACGCTAATACCGCATACGTTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTT  137
            |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  100  GAAACGGACGCTAATACCGCATACG-TCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTT  158

Query  138  GCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGC  197
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  159  GCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGC  218

Query  198  TACAATCCGTAACTGGTCTGAGAGGATGCTACAGTCACACTGGAACTGAGACACGGTCCA  257
            ||| ||||||||||||||||||||||| | ||||||||||||||||||||||||||||||
Sbjct  219  TACGATCCGTAACTGGTCTGAGAGGATGAT-CAGTCACACTGGAACTGAGACACGGTCCA  277

Query  258  GACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGAAAGCCTGATCCAGCC  317
            |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct  278  GACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCC  337

Query  318  ATGCCGCGTGTAG-GAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTGGGGGAGGAAGG  376
            |||||||||||| | ||||||||||||||||||||||||||||||| ||| |||||||
Sbjct  338  ATGCCGCGTGT-GTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGG-AGGAAGG  395

Query  377  GCATTAACCTAATACGTTAGTGTTTACGACGTTCACCGACAGAATAAGCACCGGCTAACT  436
            |||||||||||||||||||||||||  |||||| ||||||||||||||||||||||||||
Sbjct  396  GCATTAACCTAATACGTTAGTGTTTT-GACGTT-ACCGACAGAATAAGCACCGGCTAACT  453

Query  437  CTGTGCCAGCAGCCG  451
            |||||||||||||||
Sbjct  454  CTGTGCCAGCAGCCG  468
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  6   CATGCAAGTCGA  17
           ||||||||||||
Sbjct  1   CATGCAAGTCGA  12
```

159i

AGAAGGAATTGTGAGCCGGTGCTTATTCTGCAGAGTAACATCAATAGCCTAAGGTATTAACCTA
AACTACCATCTCCTCCCCTGCACAAAAGTGCTTAACAACCCGAAAGAGCCTTCCTTCCACACAC
GCCGGTATGGCTGGAATCAGGCTTCCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAG
GAGTCTGGGCCGTGTCTCAGTCCAGTGTGGCTGATCATCCTCTCAGAACAGCTAAAGATCGTCG
CCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCTACATAGGCTCATCTAATAGCGCAAGGTC
CGAAGATCCCCTGGCTTTAAAACCGTAGGACACATCCGGGTATTAGCCTAACTCTTTCCGAGTA
AGTTATCCCCAAACTTATAAGGGCAGAATCCCTATGTTTTTACCTCACCCCGTTCCGCCCACCT
CACCCATCAAAAAGAGCTAAACTTCCTCATGCCTGCCGTTCCACTGCATGTGTAAGCAAAGGAA

> gb|EU998993.1| Uncultured bacterium clone pAMS 6 16S ribosomal RNA gene, partial sequence
Length=1621
Score = 568 bits (307), Expect = 2e-158
Identities = 441/498 (89%), Gaps = 39/498 (7%)
Strand=Plus/Minus

```
Query  14   AGCCGGTGCTTATTCTGCAGAGTAACATCAATAGCCTAA-GGTATTAACCTAAACTACCA  72
            |||||||||||||||| ||| |||||||||||||| | || ||||||||||||  |||||||
Sbjct  526  AGCCGGTGCTTATTCTTCAG-GTAACATCAATAG-CAAAGGGTATTAACCTCTACTACCA  469

Query  73   TCTCCTCCCCTGCACAAAAGTGCTTAACAACCCGAAAGAGCCTTCCTTCCACACACGCCG  132
            | | || ||||| |||||||||||| |||||||| ||| ||||| ||| |||||||| ||
Sbjct  468  T-T-CT-CCCTG-ACAAAAGTGCTTTACAACCCG-AAG-GCCTT-CTT-CACACACG-CG  418

Query  133  GTATGGCTGGAATCAGGCTTCCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAG  192
            |||| ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  417  GTATTGCTGG-ATCAGGCTTCCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAG  359

Query  193  GAGTCTGGGCCGTGTCTCAGTCC-AGTGTGGCTGATCATCCTCTCAGAACAGCTAAAGAT  251
            |||||||||||||||||||||| |||||||||||||||||||||| ||||||||||
Sbjct  358  GAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAAAGAT  299

Query  252  CGTCGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCTA-CATAGGCTCATCTAATA  310
            |||||||||||||||||||||| ||||||||||||||||  |||||||||||||| |||
Sbjct  298  CGTCGCCTTGGTGAGCCTTTACCTCACCAACTAGCTAATCTTGCATAGGCTCATCTTATA  239

Query  311  GCGCAAGGTCCGAAGATCCCCTGGCTTTAAAACCGTAGGACACATCCGGGTATTAGCCTA  370
            |||||||||||||||||||||| ||||||| ||||||  ||||||||| |||||||| |
Sbjct  238  GCGCAAGGTCCGAAGATCCCCTG-CTTTAAA-CCGTAGTCCACATCCGG-TATTAGCC-A  183

Query  371  ACTCTTTCCGAGTAAGTTATCCCCAAACTTATAAGGGCAGAATCCCTATGTTTTTACCTC  430
             ||||||| ||||| |||||||| ||||| |||||| |||| ||| ||||| || || ||
Sbjct  182  -CTCTTTC-GAGTA-GTTATCCC-AAACT-ATAAGG-CAGATTCC-TATGTATT-AC-TC  132

Query  431  ACCCCGTTCCGCCCACCTCACCCATCAAAAAGAGCTAAACT-TCCTCATGCCTGCCGTTC  489
            |||| || ||||| || || || | ||||| ||| |||||| || || ||| ||||||||
Sbjct  131  ACCC-GT-CCGCC-AC-TCGCC-ACCAAAA-GAG-TAAACTCTC-TCGTGC-TGCCGTTC  81

Query  490  CACT-GCATGTGTAAGCA  506
             ||| ||||||||||||
Sbjct  80   GACTTGCATGTGTAAGCA  63
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  3   ACACATGCAAGTCGAACGG  21
           ||||||||||||||||||
Sbjct  68  ACACATGCAAGTCGAACGG  86
```

162 i

TTATTGCTGCAGATACCGTCAGCATTATCTCGGTATGTAGGGAGGATACCTTTTCTTCTCTGCCAAAAGTACTTTAC
AACCCGAAGTGCCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCAAAATTCCCCACTGCT
GCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTCA
GCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAATAGTGAGAGGTCTCGAACGATC
CCCCCCTTTCCCCCNGTATGGCGTATGCGGTATAAGCCTCTCTTTCGAGTATTGATCCCCCGCTACTGGGCACGTGT
CGATATATAACTCACCCGTGCGCCACTCGCCGCCAAAAGATGCACTGCTCTCCTCTGCTGCGTCGCACGCTTTCCGC
ACATTCGTTCACTATGTAGGGTGGAGAGCGAA

>gb|GU181289.1| Alcaligenes faecalis strain WT10 16S ribosomal RNA gene, partial sequence
Length=1536
Score = 647 bits (350), Expect = 0.0
Identities = 410/436 (95%), Gaps = 16/436 (3%)
Strand=Plus/Minus

```
Query  1    TTATTGCTGCAGATACCGTCAGCATTATCTCGGTATGTAGGGAGGATACCTTTTCTTCTC  60
            ||||| |||||||||||||||| |||||| |||| || ||| ||||||||||||||||
Sbjct  497  TTATT-CTGCAGATACCGTCAGCAGTATCTC-GTAT-TA-GGA-GATACCTTTTCTTCTC  443

Query  61   TGCCAAAAGTACTTTACAACCCGAAGTGCCTTCATCATACACGCGGGATGGCTGGATCAG  120
            |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  442  TGCCAAAAGTACTTTACAACCCGAAG-GCCTTCATCATACACGCGGGATGGCTGGATCAG  384

Query  121  GGTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  383  GGTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTC  324

Query  181  TCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTCAGCCTTGGTGAG  240
            |||||||||||||||||||||||||||||||||||||||||||||||  ||||||||||
Sbjct  323  TCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTT-GCCTTGGTGAG  265

Query  241  CCTTTACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAATAGTGAGAGGTCTCGAA  300
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  264  CCTTTACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAATAGTGAGAGGTCTTG--  207

Query  301  CGATccccccTTTCCCCCNGTATGGCGTATGCGGTATAAGCCTCTCTTTCGAGTA-TTG  359
            |||||||||||||||||| ||| |||||||||||||| |||| |||||||||||| ||
Sbjct  206  CGATCCCCCCCTTTCCCCC-GTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTT-  149

Query  360  ATCCCCCGCTACTGGGCACGTGTC-GATATATAACTCACCCGTGCGCCACTCGCCGCCAA  418
            |||||||||||||||||||| || ||||||| |||||||||| ||||||||||||||||
Sbjct  148  ATCCCCCGCTACTGGGCACGT-TCCGATATATTACTCACCCGTCCGCCACTCGCCGCCAA  90

Query  419  AAGATGCACTGCTCTC  434
             ||| |||  ||||||
Sbjct  89   GAGA-GCAA-GCTCTC  76
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  49  ACACATGCAAGTCGAACGG  67
           ||||||||||||||||||
Sbjct  3   ACACATGCAAGTCGAACGG  21
```

163 i

TTAGCTATTATTGCTGCAGATACGCTCAGCATTATCTCGGTATGTAGGGAGGATACCTTTTCTTCTCTGCCAAAAGT
ACTTTACAACCCGAAGTGACCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCAAAATTCCC
CACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGG
ATCGTCAGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAATAGTGAGAGGTCTCG
AACGATAAGCCCCTTTCCCCCGTATGGCGTATGCGGTATAAGCCTCTCTTTCGAGTATTGATCCCCGGCTACTGGGC
ACGTGTCGATATATAACTCACCCGTGCGCCACTCGCCGCCAAAAGATGCACTGCTCTCCTCTGCTGCGTCGCACGCT
TTCCGCACATTCGTTCACTATGTAGGGTGGAGA

> gb|GU181289.1| Alcaligenes faecalis strain WT10 16S ribosomal RNA gene, partial sequence
Length=1536
 Score = 614 bits (332), Expect = 2e-172
 Identities = 405/437 (93%), Gaps = 18/437 (4%)
 Strand=Plus/Minus

```
Query  8    TTATTGCTGCAGATA-CGCTCAGCATTATCTCGGTATGTAGGGAGGATACCTTTTCTTCT  66
            ||||| ||||||||| || |||||| |||||| |||| || ||| ||||||||||||||| 
Sbjct  497  TTATT-CTGCAGATACCG-TCAGCAGTATCTC-GTAT-TA-GGA-GATACCTTTTCTTCT  444

Query  67   CTGCCAAAAGTACTTTACAACCCGAAGTGACCTTCATCATACACGCGGGATGGCTGGATC  126
            |||||||||||||||||||||||||| | ||||||||||||||||||||||||||||||
Sbjct  443  CTGCCAAAAGTACTTTACAACCCGAAG-G-CCTTCATCATACACGCGGGATGGCTGGATC  386

Query  127  AGGGTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTG  186
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  385  AGGGTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTG  326

Query  187  TCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTCAGCCTTGGTG  246
            ||||||||||||||||||||||||||||||||||||||||||||||||  |||||||||
Sbjct  325  TCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTT-GCCTTGGTG  267

Query  247  AGCCTTTACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAATAGTGAGAGGTCTCG  306
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  266  AGCCTTTACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAATAGTGAGAGGTCTTG  207

Query  307  AACGATAAGCCCCTTTCCCCCGTATGGCGTATGCGGTATAAGCCTCTCTTTCGAGTA-TT  365
              ||||   |||||||||||||| |||||||||||||| |||| |||||||||||| ||
Sbjct  206  --CGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTT  149

Query  366  GATCCCCGGCTACTGGGCACGTGTC-GATATATAACTCACCCGTGCGCCACTCGCCGCCA  424
             |||||| |||||||||||||| || ||||||| |||||||||| ||||||||||||||||
Sbjct  148  -ATCCCCCGCTACTGGGCACGT-TCCGATATATTACTCACCCGTCCGCCACTCGCCGCCA  91

Query  425  AAAGATGCACTGCTCTC  441
            | ||| |||  ||||||
Sbjct  90   AGAGA-GCAA-GCTCTC  76
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  49  ACACATGCAAGTCGAACGG  67
           ||||||||||||||||||| 
Sbjct  3   ACACATGCAAGTCGAACGG  21
```

166 i

GCATGAGAAGGAATTGTGAGCCGGTGCTTATTCTGCAGAGTAACATCAATAGCCTAAGGTATTAACCTAAACTACCATCTCCGC
CCCCTGCACAAAAGTGCTTAACAACCCGAAAGAGCCTTCCTTCCACACACGCCGGTATGGCTGGAATCAGGCTTCCGCCCATTG
TCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCAGTGTGGCTGATCATCCTAGCAGAACAGCTA
AAGATCGTCGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCTACATAGGCTCATCTAATAGCGCAAGGTCCGAAGATCCC
CTGGCTTTAAAACCGTAGGACACATCCGGGTATTAGCCTAACTCTTTCCGAGTAAGTTATCCCCAAACTTATAAGGGCAGAATC
CCTATGTTTTTACCTCACCCCGTTCCGCGCAACCTCACCCATCAAAAAGAGCTAAACTTCCTCATGCCTGCCGTTTTACTGCATGT
GTAAGCAAAGGAAGCTA

> gb|EU998993.1| Uncultured bacterium clone pAMS 6 16S ribosomal RNA gene, partial sequence
Length=1621 Score = 536 bits (290), Expect = 5e-149
Identities = 437/500 (88%), Gaps = 41/500 (8%)
Strand=Plus/Minus

```
Query  19   AGCCGGTGCTTATTCTGCAGAGTAACATCAATAGCCTAA-GGTATTAACCTAAACTACCA  77
            |||||||||||||||| ||| |||||||||||||| | || ||||||||||||  |||||||
Sbjct  526  AGCCGGTGCTTATTCTTCAG-GTAACATCAATAG-CAAAGGGTATTAACCTCTACTACCA  469

Query  78   TCTCCGCCCCCTGCACAAAAGTGCTTAACAACCCGAAAGAGCCTTCCTTCCACACACGCC  137
            | |   | ||||| |||||||||||| |||||||| ||| ||||| ||| |||||||| |
Sbjct  468  T-T---CTCCCTG-ACAAAAGTGCTTTACAACCCG-AAG-GCCTT-CTT-CACACACG-C  419

Query  138  GGTATGGCTGGAATCAGGCTTCCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTA  197
            ||||| ||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  418  GGTATTGCTGG-ATCAGGCTTCCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTA  360

Query  198  GGAGTCTGGGCCGTGTCTCAGTCC-AGTGTGGCTGATCATCCTAGCAGAACAGCTAAAGA  256
            |||||||||||||||||||||||| ||||||||||||||||  |||| ||||||||||
Sbjct  359  GGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCAGACCAGCTAAAGA  300

Query  257  TCGTCGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCTA-CATAGGCTCATCTAAT  315
            ||||||||||||||||||||||||| ||||||||||||||||  |||||||||||||| ||
Sbjct  299  TCGTCGCCTTGGTGAGCCTTTACCTCACCAACTAGCTAATCTTGCATAGGCTCATCTTAT  240

Query  316  AGCGCAAGGTCCGAAGATCCCCTGGCTTTAAAACCGTAGGACACATCCGGGTATTAGCCT  375
            ||||||||||||||||||||||| ||||||| ||||||  ||||||||| ||||||||
Sbjct  239  AGCGCAAGGTCCGAAGATCCCCTG-CTTTAAA-CCGTAGTCCACATCCGG-TATTAGCC-  184

Query  376  AACTCTTTCCGAGTAAGTTATCCCCAAACTTATAAGGGCAGAATCCCTATGTTTTTACCT  435
            | ||||||| ||||| |||||||| ||||| |||||| |||| ||| ||||| || || |
Sbjct  183  A-CTCTTTC-GAGTA-GTTATCCC-AAACT-ATAAGG-CAGATTCC-TATGTATT-AC-T  133

Query  436  CACCCCGTTCCGCGCAACCTCACCCATCAAAAAGAGCTAAACT-TCCTCATGCCTGCCGT  494
            ||||| || |||| || | || || | ||||| ||| |||||| || || ||| ||||||
Sbjct  132  CACCC-GT-CCGC-CA-C-TCGCC-ACCAAAA-GAG-TAAACTCTC-TCGTGC-TGCCGT  83

Query  495  TTTACT-GCATGTGTAAGCA  513
            |  ||| |||||||||||||
Sbjct  82   TCGACTTGCATGTGTAAGCA  63
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  3   ACACATGCAAGTCGAACGG  21
           ||||||||||||||||||
Sbjct  68  ACACATGCAAGTCGAACGG  86
```

169 i

TGATTGCTGGTCGAGTAACGTGCAAAACAGTCAAATGATGTAGTGTAACTGCCCTTCCTCCCAACTTAAAGTGCTTT
ACAATCCTAAGAGCCTTCTTCACACACGCGGCATGGCTGGATGCAGGGGTTTCCCCCATTGTCCAATATTCCCCACT
GCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCG
CGGTCTTGGTGAGCCATTACCTCACCAACTAACTAATCCGACCTAGGCTCATCTAATAGCGAAAGGCTCCAAAGAG
TCCCCTCCTTTCTCCCGTAGGACGTATGCGGTATTAGCTTACCTTTCGGCAAGTTATCCCCCACTACTAGGGCAGAT
TCCTAGGCATTACTCACCCGTCCGCCGCTCGTCAGCAAAGAAGCAAGCTTCTTCCTGTTACCGTTCGACTTGCATGT
GTAAAG

> gb|AY017062.1|AY017062S1 Pseudomonas sp. CL-2 16S ribosomal RNA gene, 5'-partial sequence
Length=816
Score = 758 bits (410), Expect = 0.0
Identities = 443/457 (97%), Gaps = 10/457 (2%)
Strand=Plus/Minus

```
Query  10   GTCGAGTAACGTGCAAAACAGTCAAATGATGTAGTGTAACTGCCCTTCCTCCCAACTTAA  69
            |||| ||||||| ||||||||||||| || |||| ||||||| ||||||||||||||||
Sbjct  493  GTCG-GTAACGT-CAAAACAGTCAAAT-AT-TAGT-TAACTGCTCTTCCTCCCAACTTAA  439

Query  70   AGTGCTTTACAATCCTAAGAGCCTTCTTCACACACGCGGCATGGCTGGATGCAGGGGTTT  129
            |||||||||||||||||| |||||||||||||||||||||||| || ||||||
Sbjct  438  AGTGCTTTACAATCCTAAGA-CCTTCTTCACACACGCGGCATGGCTGGAT-CA-GGGTTT  382

Query  130  CCCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGT  189
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  381  CCCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGT  322

Query  190  TCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGCGGTCTTGGTGAGCCATTA  249
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  321  TCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGCGGTCTTGGTGAGCCATTA  262

Query  250  CCTCACCAACTAACTAATCCGACCTAGGCTCATCTAATAGCGAAAGGCTCCAAAGAGTCC  309
            |||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Sbjct  261  CCTCACCAACTAACTAATCCGACCTAGGCTCATCTAATAGCGAAAGGCTCCGAAGAGTCC  202

Query  310  CCTCCTTTCTCCCGTAGGACGTATGCGGTATTAGCTTACCTTTCGGCAAGTTATCCCCCA  369
            |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  201  CCTCCTTTCTCCCGTAGGACGTATGCGGTAT-AGCTTACCTTTCGGCAAGTTATCCCCCA  143

Query  370  CTACTAGGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTCGTCAGCAAAGAAGCAA  429
            |||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  142  CTACTAGG-CAGATTCCTAGGCATTACTCACCCGTCCGCCGCTCGTCAGCAAAGAAGCAA  84

Query  430  GCTTCTTCCTGTTACCGTTCGACTTGCATGTGTAAAG  466
            |||||||||||||||| |||||||||||||| |||
Sbjct  83   GCTTCTTCCTGTTACCGCTCGACTTGCATGTGTTAAG  47
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  49  TAACACATGCAAGTCGAGCGG  69
           |||||||||||||||| |||
Sbjct  1   TAACACATGCAAGTCGAACGG  21
```

173 i

CGATTTATTCTGTCGGTAACGTCAAAACACTAACGTATTAGGTTAATGCCCTTCCTCCCAACTTAAAGTGCTTTACA
ATCCGAAGACCTACTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCTTTGTCCAATATTCCCCACTGCTGCC
TCCCGTAGGAGTCTCGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTT
GGCGAGCCATTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCT
TTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCCCCCACTACCAGGCAGATTCCTAGGTAT
TACTCACCCGTCCGCCGCTCTCAAGAGGTGCAAGCACCTCTCTACCGTTCGACTTGCATGTGTAAGGACAGAAC

> gb|HM332859.1| Uncultured bacterium clone ncd1064f04c1 16S ribosomal RNA gene, partial sequence
Length=1358
Score = 793 bits (429), Expect = 0.0
Identities = 441/447 (99%), Gaps = 0/447 (0%)
Strand=Plus/Minus

```
Query  5    TTATTCTGTCGGTAACGTCAAAACACTAACGTATTAGGTTAATGCCCTTCCTCCCAACTT  64
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  466  TTATTCTGTCGGTAACGTCAAAACACTAACGTATTAGGTTAATGCCCTTCCTCCCAACTT  407

Query  65   AAAGTGCTTTACAATCCGAAGACCTACTTCACACACGCGGCATGGCTGGATCAGGCTTTC  124
            |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  406  AAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTC  347

Query  125  GCCCTTTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTCGACCGTGTCTCAGTT  184
            |||| ||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  346  GCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTT  287

Query  185  CCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGCGAGCCATTAC  244
            |||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  286  CCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTAGCCTTGGTGAGCCATTAC  227

Query  245  CTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCT  304
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  226  CTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCT  167

Query  305  GCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCCCCCACTA  364
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  166  GCTTTCTCCCGTAGGACGTATGCGGTATTAGCGTCCGTTTCCGAACGTTATCCCCCACTA  107

Query  365  CCAGGCAGATTCCTAGGTATTACTCACCCGTCCGCCGCTCTCAAGAGGTGCAAGCACCTC  424
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  106  CCAGGCAGATTCCTAGGTATTACTCACCCGTCCGCCGCTCTCAAGAGGTGCAAGCACCTC  47

Query  425  TCTACCGTTCGACTTGCATGTGTAAGG  451
            ||||||| ||||||||||||||| |||
Sbjct  46   TCTACCGCTCGACTTGCATGTGTTAGG  20
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  1   TAACACATGCAAGTCGA  17
           |||||||||||||||||
Sbjct  22  TAACACATGCAAGTCGA  38
```

175i

TTTTCTGCAAGTAACGTCATTATCTTCCTTGCTAAAAGAAGCTTTACAACCCTAAGGCCTTCATCACTCACTCGGTAT
GTGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGT
CCCAGTGTGGCTGATCATCCTCTCAGACCAGCTACAGATCGTCGGCTTGGTGAGCCGTTACCTCACCAACTACCTA
ATCTGACACGGGCTCATCCATCAGCGATAAATCTTTCCTCCGTAGAGAATATACGGTATTAGCTTTTATTTCTAAAA
GTTATTCCGTACTGATGGGCAGATTCCCACGTGTTACTCACCCGTCTGCCACTAACTAATTGGAGCAAGCCCCAATT
AGTCCGTTCGACTTGCATGTGTAAAGCAAAGAG

> gb|AY961085.1| Rickettsia endosymbiont of Coccotrypes dactyliperda 16S ribosomal RNA gene, partial sequence
Length=1454

Score = 734 bits (397), Expect = 0.0
Identities = 408/413 (99%), Gaps = 2/413 (0%)
Strand=Plus/Minus

```
Query  1    TTTTCTGCAAGTAACGTCATTATCTTCCTTGCTAAAAGAAGCTTTACAACCCTAAGGCCT  60
            |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||
Sbjct  438  TTTTCTGCAAGTAACGTCATTATCTTCCTTGCTAAAAG-AGCTTTACAACCCTAAGGCCT  380

Query  61   TCATCACTCACTCGGTATGTGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACT  120
            |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  379  TCATCACTCACTCGGTAT-TGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACT  321

Query  121  GCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  320  GCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATCCTCTCA  261

Query  181  GACCAGCTACAGATCGTCGGCTTGGTGAGCCGTTACCTCACCAACTACCTAATCTGACAC  240
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  260  GACCAGCTACAGATCGTCGGCTTGGTGAGCCGTTACCTCACCAACTACCTAATCTGACGC  201

Query  241  GGGCTCATCCATCAGCGATAAATCTTTCCTCCGTAGAGAATATACGGTATTAGCTTTTAT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  200  GGGCTCATCCATCAGCGATAAATCTTTCCTCCGTAGAGAATATACGGTATTAGCTTTTAT  141

Query  301  TTCTAAAAGTTATTCCGTACTGATGGGCAGATTCCCACGTGTTACTCACCCGTCTGCCAC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  140  TTCTAAAAGTTATTCCGTACTGATGGGCAGATTCCCACGTGTTACTCACCCGTCTGCCAC  81

Query  361  TAACTAATTGGAGCAAGCCCCAATTAGTCCGTTCGACTTGCATGTGTAAAGCA  413
            |||||||||||||||||||||||||||||||| ||||||||||||| |||||
Sbjct  80   TAACTAATTGGAGCAAGCCCCAATTAGTCCGTCCGACTTGCATGTGTTAAGCA  28
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  32  TAACACATGCAAGTCGGACGGA  53
           |||||||||||||||| |||||
Sbjct  1   TAACACATGCAAGTCGAACGGA  22
```

180i

GTTCAAACATATCACTACATCGTTATTAGAAGTACGGATAGACCCACTGGCTAAGCTAAGCCTCAAAAGTGCTTTA
CAATCCGAAGACGTTCTATCACTACACGCGGGATGGCTGGATCAGGCGTTGTCGCCCATTGTCCAATATTCCCCAC
TGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGCTACGGATC
ATCGCCTTGGTGAGCCATTACCCCACCAACTAGCTAATCCGATCTAGGCTCATCTAATAGCGCAAGGTCCGAAGGT
CCCCCGGTTTTGCCCGTAGGACGTATGCGGTATTAGCGTCTCTTTCGAGAAGTTATCCCCCACTACCGGGCACATAC
CTATGCATTACTCACCCGTCCGCCACTCAACTGCAAAAACACGCACCTCTCACCCCGCCTGGCACGATTGCGAATG
GTGTTAATTGTGGCTATAAAAGAAACACCAAAAAGGAACAAG

>gb|GU300357.1| Uncultured Pseudomonas sp. clone PSB011.C21_E13 16S ribosomal
RNA gene, partial sequence
Length=794

 Score = 503 bits (272), Expect = 4e-139
 Identities = 319/341 (94%), Gaps = 6/341 (1%)
 Strand=Plus/Minus

```
Query  66   AAAGTGCTTTACAATCCGAAGACGTTCTATCACTACACGCGGGATGGCTGGATCAGGCGT  125
            ||||||||||||||||||||||| |||| |||| |||||||| ||||||||||||||| |
Sbjct  389  AAAGTGCTTTACAATCCGAAGACCTTCT-TCAC-ACACGCGGCATGGCTGGATCAGGC-T  333

Query  126  TGTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTC  185
            | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  332  T-TCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTC  274

Query  186  AGTTCCAGTGTGACTGATCATCCTCTCAGACCAGCTACGGATCATCGCCTTGGTGAGCCA  245
            |||||||||||||||||||||||||||||||| |||||||| ||||||||||||||||
Sbjct  273  AGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCCA  214

Query  246  TTACCCCACCAACTAGCTAATCCGATCTAGGCTCATCTAATAGCGCAAGGTCCGAAGGTC  305
            |||||||||||||||||||||||| |||||||||||| |||||||||| |||||||||
Sbjct  213  TTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCAAGGCCCGAAGGTC  154

Query  306  CCCCGGTTT-TGCCCGTAGGACGTATGCGGTATTAGCGTCTCTTTCGAGAAGTTATCCCC  364
            ||| | ||| | |||||||||||||||||||||||||||| ||||||||| ||| |||||
Sbjct  153  CCCTGCTTTCT-CCCGTAGGACGTATGCGGTATTAGCGTCCCTTTCGAGACGTTGTCCCC  95

Query  365  CACTACCGGGCACATACCTATGCATTACTCACCCGTCCGCC  405
            ||||||| |||| || |||| ||||||||||||||||||||
Sbjct  94   CACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCC  54
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

```
Query  1   TAACACATGCAAGTCGAACGGA  22
           |||||||||||||||| ||||
Sbjct  5   TAACACATGCAAGTCGAGCGGA  26
```

211 i

TGATTGCTGGTCGAGTAACGTGCAAAACAGTCAAATGATGTAGTGTAACTGCCCTTCCTCCCAACTTAAAGTGCTTT
ACAATCCTAAGAGCCTTCTTCACACACGCGGCATGGCTGGATGCAGGGGTTTCCCCCATTGTCCAATATTCCCCACT
GCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCG
CGGTCTTGGTGAGCCATTACCTCACCAACTAACTAATCCGACCTAGGCTCATCTAATAGCGAAAGGCTCCAAAGAG
TCCCCTCCTTTCTCCCGTAGGACGTATGCGGTATTAGCTTACCTTTCGGCAAGTTATCCCCCACTACTAGGGCAGAT
TCCTAGGCATTACTCACCCGTCCGCCGCTCGTCAGCAAAGAAGCAAGCTTCTTCCTGTTACCGTTCGACTTGCATGT
GTAAAG

> gb|AY017062.1|AY017062S1 Pseudomonas sp. CL-2 16S ribosomal RNA gene, 5'-partial sequence
Length=816
 Score = 758 bits (410), Expect = 0.0
 Identities = 443/457 (97%), Gaps = 10/457 (2%)
 Strand=Plus/Minus

```
Query  10   GTCGAGTAACGTGCAAAACAGTCAAATGATGTAGTGTAACTGCCCTTCCTCCCAACTTAA  69
            |||| ||||||| ||||||||||||||| || |||| ||||||| ||||||||||||||||
Sbjct  493  GTCG-GTAACGT-CAAAACAGTCAAAT-AT-TAGT-TAACTGCTCTTCCTCCCAACTTAA  439

Query  70   AGTGCTTTACAATCCTAAGAGCCTTCTTCACACACGCGGCATGGCTGGATGCAGGGGTTT  129
            |||||||||||||||||||| |||||||||||||||||||||||||||| || ||||||
Sbjct  438  AGTGCTTTACAATCCTAAGA-CCTTCTTCACACACGCGGCATGGCTGGAT-CA-GGGTTT  382

Query  130  CCCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGT  189
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  381  CCCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGT  322

Query  190  TCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGCGGTCTTGGTGAGCCATTA  249
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  321  TCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGCGGTCTTGGTGAGCCATTA  262

Query  250  CCTCACCAACTAACTAATCCGACCTAGGCTCATCTAATAGCGAAAGGCTCCAAAGAGTCC  309
            |||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Sbjct  261  CCTCACCAACTAACTAATCCGACCTAGGCTCATCTAATAGCGAAAGGCTCCGAAGAGTCC  202

Query  310  CCTCCTTTCTCCCGTAGGACGTATGCGGTATTAGCTTACCTTTCGGCAAGTTATCCCCCA  369
            |||||||||||||||||||||||||||||| |||||||||||||||||||||||||||||
Sbjct  201  CCTCCTTTCTCCCGTAGGACGTATGCGGTAT-AGCTTACCTTTCGGCAAGTTATCCCCCA  143

Query  370  CTACTAGGGCAGATTCCTAGGCATTACTCACCCGTCCGCCGCTCGTCAGCAAAGAAGCAA  429
            |||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  142  CTACTAGG-CAGATTCCTAGGCATTACTCACCCGTCCGCCGCTCGTCAGCAAAGAAGCAA  84

Query  430  GCTTCTTCCTGTTACCGTTCGACTTGCATGTGTAAAG  466
            |||||||||||||||| ||||||||||||||| |||
Sbjct  83   GCTTCTTCCTGTTACCGCTCGACTTGCATGTGTTAAG  47
```

Alignment with *Ehrlichia/Anaplasma* TaqMan common probe

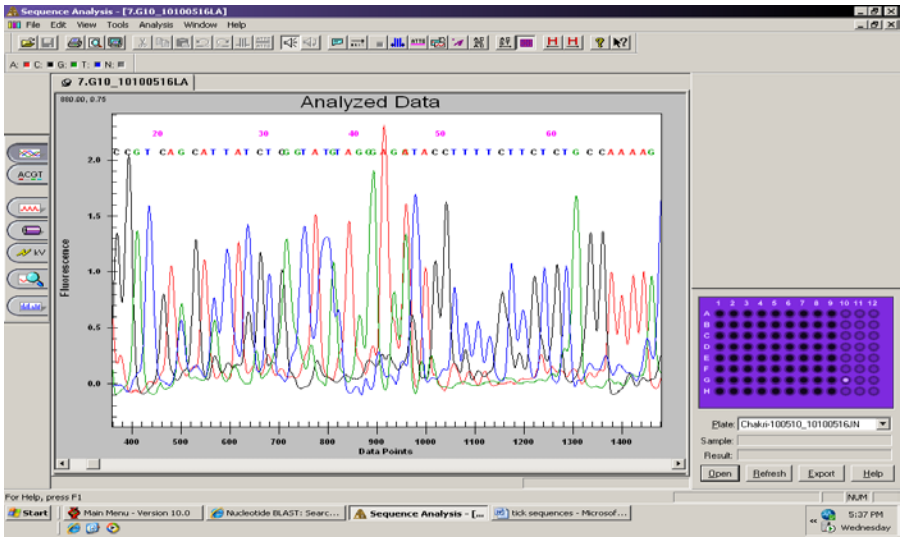
```
Query  49  TAACACATGCAAGTCGAGCGG  69
           |||||||||||||||| |||
Sbjct  1   TAACACATGCAAGTCGAACGG  21
```

Table 7. The list of species that are identified by tick DNA samples

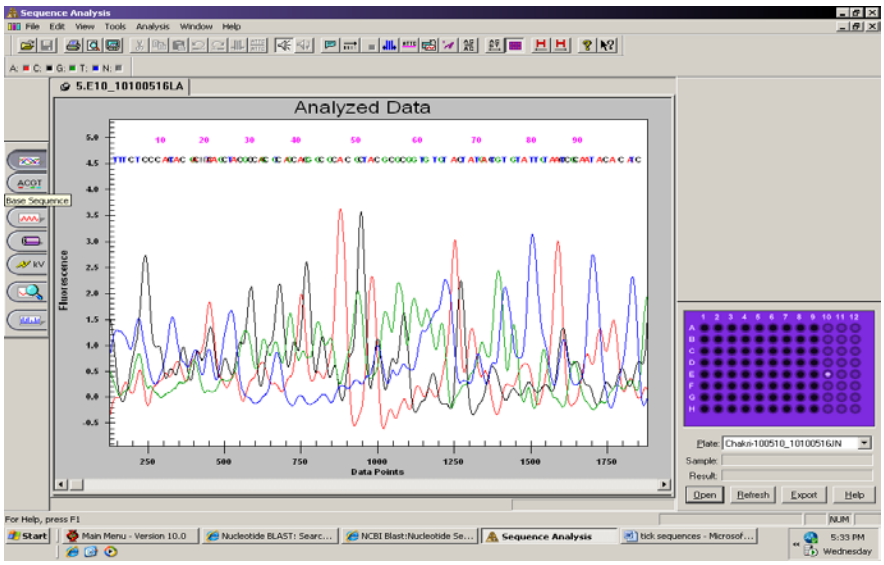
Organism name	Sample Id	No.of nucleotide difference with *Ehrlichia/Anaplasma* common TaqMan probe	No.of nucleotide difference with *Ehrlichia/Anaplasma* common Forward primer	No.of nucleotide difference with *Ehrlichia/Anaplasma* common Reverese primer
gb|DQ158108.1| Uncultured bacterium clone 516	24i	1	5	0
gb|HM069816.1| Uncultured bacterium clone Bacteria_Clone_349	27i	2	9	0
gb|EU998993.1| Uncultured bacterium clone pAMS 6	159i 166i	3	8	0
gb|HM561497.1| Uncultured Pseudomonas sp. clone Dn12	48i 144i	5	5	0
gb|GU062533.1| Pseudomonas sp. KOPRI 25416	157i	10	13	0
gb|HM332859.1| Uncultured bacterium clone ncd1064f04c1	54i 173i	5	5	0
gb|AY017062.1|AY017062S1 Pseudomonas sp. CL-2	105i 169i 211i	2	9	0
gb|EF192826.1| Uncultured bacterium clone IM4_H04	112i	7	11	0
gb|FJ950669.1| Pseudomonas sp. d130	124i	15	18	0
gb|GU181289.1| Alcaligenes faecalis strain WT10	131A 162i 163i	3	8	0
gb|GU300357.1| Uncultured Pseudomonas sp. Clone PSB011.C21_E13	152i 180i	1	5	0
gb|AY961085.1| Rickettsia endosymbiont of Coccotrypes dactyliperda	175i	1	9	3

Fig 9. Fluorescence emission peaks diagrams of DNA sequence analysis generated in Beckman Coulter CEQ 8000 Genetic Analysis System.

a)



b)



The figure represents the sequencing readouts of two randomly selected samples which contain homogenous and heterogenous mixes of PCR products. The panel 'A' has clean sequence readouts, suggesting the PCR DNA is homogenous.The panel 'B' shows multiple overlapping peaks suggesting the PCR DNA is heterogenous.

Discussion:

Studies over the last three decades led to the identification of many newly discovered tick transmitted infections in people and animals. They include *E. chaffeensis* (HME agent) and *E. ewingii* (HEE agent) transmitted by *A. americanum* tick; *A. phagocytophilum* (HGA agent) (66, 68) and *B. burgdorferi* (Lyme disease agent) transmitted by *I. scapularis* tick (24, 64). Similarly, several tick transmitted *Rickettsia* species are identified as the pathogens of people. These include *Rickettsia parkeri* infection discovered in 1990 which is transmitted by *A. maculatum* (Gulf Coast tick) (75). Tick transmitted *Ehrlichia, Anaplasma* and *Rickettsia* species infections are classified as the emerging diseases, because of the rapid increase in documented cases since their initial discoveries. The tick-borne rickettsial diseases are also responsible for severe and potentially fatal diseases in animals. Interestingly, little is known about how the rickettsial agents are adapted to humans in causing diseases. Until the first discovery of *E. chaffeensis* in 1986, *Ehrlichia* and *Anaplasma* species are not considered as human pathogens. It is now an established fact that *E. chaffeensis, E. ewingii* and *A. phagocytophilum* are important agents of tick-borne diseases in people in the USA and many parts of the world (64-68, 71). However, little is known about their existence in the nature prior to their first documented human cases. The organisms may exist in wildlife and ticks and were not pathogens of humans till recently. Secondly, due to increased exposure of humans to a closer proximity of nature where tick exposure increases may result in the human infections from tick bites. Alternatively, that the co-existence of several non-pathogenic and pathogenic microorganisms with in a reservoir host and in a tick may provide opportunities for changes in the genomes of several bacteria as a result of genetic exchanges among closely related bacteria. These genomic changes may contribute to the generation of pathogenic organisms.

In this study, we assessed for the presence of various *Ehrlichia* and *Anaplasma* species in a reservoir host, white-tailed deer, and in ticks harboring of ticks. Our experimental approach included evaluating 147 deer blood samples for the presence of *Ehrlichia* and *Anaplasma* species by performing real time TaqMan probe based PCR and RT-PCR analyses. Similar experiments were also performed on 37 tick pools collected from the deer. Seventy four percent of the deer samples (113 out of 147 samples) tested positive for the presence of *Ehrlichia* and *Anaplasma* species. To determine the identity of *Ehrlichia* species, we used *E. chaffeensis*- and *E. ewingii*-specific probes and repeated the real time RT-PCR and PCR assays. These analyses identified fewer positives for these two species (3% for *E. chaffeensis* and 5% for *E. ewingii*). We did not assess for the presence of *Anaplasma* species in deer blood because deer is not considered as a reservoir host for *A. phagocytophilum* or *A. marginale*. It is possible that deer may carry these and other *Ehrlichia* and *Anaplasma* species. Deer blood derived nucleic acids were utilized in a PCR or RT-PCR assays using a PCR primer set that is expected to amplify a 16S rDNA segment for any known *Ehrlichia* and *Anaplasma* species. These experiments resulted in 26 samples (Out of 113 samples analyzed) (23%) positives for 16S rRNA gene segment. Sequence analysis of the 24 amplicons led to the identifying of one unnamed *Ehrlichia* species commonly found in white-tailed deer, which regarded as the *Ehrlichia* species GA isolate No. 4 (Genebank # gb|U27104.1|ESU27104) (74). The second closest homology identified for these sequences is with *Anaplasma* species WTD 81 isolate (Genebank # gb|DQ007352.1|). These two species are nearly identical for the entire 16S rDNA sequence except for two nucleotide differences and probably they may represent DNA from the same species. Previous study on deer blood analysis also identified white-tailed deer isolate; *Ehrlichia* species GA isolate No. 4 to be present in all 10 samples analyzed (74). Our results for the identification of *Ehrlichia*

species GA isolate No. 4 are consistent with the data reported in the literature. However, in this test study more samples were evaluated. The positives in deer blood identified are relatively less (74%) compared to a previous report of the 100% of the animals being the *Ehrlichia* species GA isolate No. 4. This difference may be due to large number of samples analyzed in the present study. Moreover, there may be geographical variation in the prevalence of *Ehrlichia* species GA isolate No. 4 we collected. The pathogenic potential of *Ehrlichia* species GA isolates No. 4 to humans or other vertebrate animals remain to be established. The presence of a closely related *Ehrlichia* species similar to pathogenic organisms, *E. chaffeensis* and *E. ewingii*, support the possibility that the organisms co-evolve within a reservoir host and may aid in the adaptation to new hosts such as humans.

Molecular survey for the presence of *Ehrlichia* and *Anaplasma* species in ticks also identified large numbers of real time PCR positives with *Ehrlichia* and *Anaplasma* species common TaqMan probe (29 out of 37 samples) (78%). *E. chaffeensis* and *E. ewingii* specific probes in real time PCR assays identified fewer numbers of positives. The presence of higher numbers of *Ehrlichia* and *Anaplasma* species positives is very similar to the data obtained for deer blood. Sequence analyses of a segment of 16S rDNA, however, did not identify any *Ehrlichia* or *Anaplasma* species. The PCR assays with *Ehrlichia* and *Anaplasma* species specific PCR primer set, however, yielded significantly more positives (33 out of 37 samples) (about 90%). The sequence analysis identified Gram-negative bacteria species which included one endosymbiont of *Rickettsia* species (one tick pool), one *Alcaligenes faecalis* strain (three tick pools), five different *Pseudomonas* species (9 tick pools) and five different uncultured bacteria organisms (7 tick pools). None of the samples analyzed by sequencing included *Ehrlichia* and

Anaplasma species. The high number of PCR positives and yet the absence of *Ehrlichia* and *Anaplasma* species in the sequences analyzed is unexpected.

Ticks are known to harbor large numbers of bacteria in them. For example, a recent study reported the presence of 151 bacterial isolates in ticks (67 strains from *Ixodes ricinus*, 38 from *Dermacentor reticulatus*, 46 strains from *Haemaphysalis concinna*) (76). Similarly, another study reported the presence of several bacterial species (including pathogenic species) belonging to genera *Rickettsia*, *Pseudomonas*, *Borrelia*, *Ralstonia*, *Anaplasma*, *Enterobacterias*, *Moraxella*, *Rhodococcus* and uncultured proteobacterium in *I. scapularis* ticks (77). It is evident from these studies that ticks serve as host for numerous bacteria both pathogenic and non-pathogenic. Our careful analysis of the DNA sequence profile analyzed from the fluorescent peaks identified by the DNA sequences suggested that the tick derived 16S rDNA sequences included multiple overlapping peaks. These data suggest that the amplicons may be complex and the sequences identified may not represent all different species of bacteria present in ticks. Importantly, ticks we analyzed may have included numerous bacteria including *Ehrlichia* and *Anaplasma* species identified by real time TaqMan based PCR assays.

This study demonstrated that tick and white-tailed deer harbor *Ehrlichia* and *Anaplasma* species in large numbers. High percentage of these positives represents non-pathogenic *Ehrlichia* species. Similarly, most of the ticks analyzed are also positive for *Ehrlichia* and *Anaplasma* species as judged from the real time TaqMan based PCR. Inaddition, ticks also contained numerous bacterial organisms. The presence of multiple species of bacteria within ticks and reservoir hosts support our working hypothesis that the co-existence of several non-pathogenic and pathogenic microorganisms within reservoir hosts and in ticks may provide opportunities for changes in the genomes in several bacteria as a result of genetic exchanges. It

is, however, not clear that the presence of multiple bacterial organisms in ticks and reservoir hosts contribute to the generation of novel species pathogenic to humans and other vertebrate animals. Similarly, the pathogenic potential of numerous bacteria, including *Ehrlichia* and *Anaplasma* species present in ticks and white-tailed deer remains to be determined.

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