

DNA Based Diagnosis of Canine Bacterial Diseases

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Abstract: In current era pets are important part of the social life. Moreover, increased use of animal assisted therapies in treatment of people with special needs gives a glimpse of their beneficial role in future. Dogs are one of the commonly adopted animals as pet and more often develops an intimate relation with their owners. Hence, chances of disease transfer from dogs to humans are high. In this review some important bacterial diseases of dogs are discussed with respect to their DNA based diagnosis. PCR was found to be effective for detection of diseases in most of the cases. To distinguish between pathogenic and nonpathogenic strains of an organism, combination of PCR with other DNA based diagnostic techniques like Restriction Fragment Length Polymorphism (RFLP), found to be effective. In future continuous efforts are required to devise new methods for rapid detection of these pathogens.

Key words: Brucellosis, colibacillosis, giardiasis, Lyme, leptospirosis, salmonellosis, campylobacteriosis, urinary tract infection

INTRODUCTION

The relationship between humans and their animals is very old and these days pets are becoming more common at homes as a social substitute. Pets are more commonly associated with induction of well being in people and development of social skills in children. On an average about 62% of US households have a pet, which shows that these pets are integral part of the daily social life (APPA, 2011). People suffering with loneliness are often advised to adopt a pet to reduce the harmful effects of social loneliness. In addition to all these factors adaptation of animal assisted therapies in treatment of people with special needs results in elevated number of pets.

Increased number of pets in the daily life also increases the risk infections that are zoonotic in nature. Brucellosis is a common disease of animals (Abd El-Razik *et al.*, 2007; Junaidu *et al.*, 2006) and it is also reported in humans (Hedayatizadeh-Omran *et al.*, 2010; Akhtar and Latif, 2000). *Dirofilaria immitis*, *Ehrlichia canis* and *Borrelia burgdorferi* are pathogens that are known for causing infections in both animals and humans (Melek *et al.*, 2005; Karami *et al.*, 2006; Ranjbar-Bahadori *et al.*, 2007; Icen *et al.*, 2011; Gholami *et al.*, 2011). Echinococcosis is a common disease reported in various animals, it is also common in humans; Vahedi and Vahedi (2012) conducted a study in Iran to

check its prevalence in humans, they concluded that this disease was mainly found in females (age 20-40). In addition, liver and lungs were found to be the major sites of infection. Influenza virus is known for causing infection in birds Majidzadeh *et al.* (2011), Farooq *et al.* (2006) and Hadipour (2011) has reported that poultry farm workers and veterinarians are highly prone to this disease. All these facts highlight the importance of proper detection and treatment of these animal diseases, not only for the improvement of animal health but also for humans' welfare.

Development of resistance in these pathogens against currently used antimicrobial drugs is a potential threat to human health (Moniri and Dastehgoli, 2007; Karou *et al.*, 2009; Joseph *et al.*, 2011; Mehrabian and Jaber, 2007). The emergence rate of antimicrobial resistant pathogens is higher than the discovery of new drugs that are effective against these pathogens; this fact leads the scientists to find new sources for antifungal (Malabadi and Kumar, 2007; Karthishwaran and Mirunalini, 2010; Widodo *et al.*, 2008; Hassan *et al.*, 2007; Hadizadeh *et al.*, 2009; Harun-Or-Rashid *et al.*, 2006) antiviral (Sohail *et al.*, 2011a, b; Vignesh *et al.*, 2011; Momtaz and Abdollahi, 2010) and antibacterial agents (Jazani *et al.*, 2008; Anam *et al.*, 2010; Haque *et al.*, 2009; Geidam *et al.*, 2007; Manikandan *et al.*, 2011; Motamedi *et al.*, 2010). These days plants are under extensive research to identify new compounds that have

potential medicinal properties (Karim *et al.*, 2011; Sohail and Sohail, 2011; Sohail *et al.*, 2011c; Karim and Kanwal, 2011; Ahmida, 2011; Oyedemi *et al.*, 2011; Iweala *et al.*, 2011) and many potential antimicrobial compounds have been identified. However, suppression of factors that promote antimicrobial resistance will be helpful in delaying the emergence of antimicrobial resistance. Domestic animals that are taking antibiotics either directly or indirectly have been reported as a source of antibiotic resistant pathogens (Leonard *et al.*, 2012; Ahmed *et al.*, 2012). Early detection of pathogenic diseases will facilitate the effective control of pathogen and reduces the overuse of antibiotics. PCR is a good diagnostic technique for in time detection of diseases that are of zoonotic origin (Rahimi and Doosti, 2012). Researchers have conducted a literature review to highlight the importance of PCR in diagnosis of bacterial diseases of dogs, which are of zoonotic nature.

BACTERIAL DISEASES IN DOGS

Brucellosis: Brucellosis is a bacterial infection in animals, which is caused by a Gram-ve bacteria called *Brucella canis* (Moore, 1969). It is the most common zoonotic diseases, which was listed as a human affecting disease in the early 20th century on Malta island and hence known as Malta fever. This disease can affect almost all domestic animals and it is more common in countries with poor standards of animal and public health programs (Capasso, 2002). Prevalence of this disease varies among animals, e.g., cattle (0.85-23.3%), buffaloes (4.2-10%) camels (0.0-17.20%), goat (0.86-12%) and many others (Ahmed and Munir, 1995; Refai, 1989; Memish, 2001; El-Ansary *et al.*, 2001; Al-Ani *et al.*, 1998; Cadmus *et al.*, 2006; Refai, 2000). It is believed that this disease is more common in males as compared to females (Hussein *et al.*, 2005) however, there are some reports claiming that the prevalence of this disease is independent of animal's sex (Muma *et al.*, 2006). It mainly affects the mature cows as Amin *et al.* (2005) reported that animals having age above 4 years are more prone to this infection.

In dogs the primarily signs of brucellosis are abortion, scrotal inflammation and orchitis. Contact with aborted fetus transmits disease from one to another, which results in damage to genitals and lymphatic system. This disease can be studied by various serological and bacteriological techniques, among those Tube Agglutination Test (TAT) is more common (Pickerill and Carmichael, 1972). A study was conducted by Flores-Castro *et al.* (1977) to determine canine brucellosis of naturally infected dogs in Mexico city. They diagnosed the disease using both serological and bacteriological methods and found a very high rate of *Brucella canis* infection in stray dogs (11.8%) of Mexico

city. DNA based detection techniques are very successful in its diagnosis; in a study researchers from Italy used whole blood and buffy coat of infected animals to successfully detect the pathogenic organism through real-time PCR (Corrente *et al.*, 2010). They reported the detection of a brucellosis positive case through real-time PCR, which was previously not detectable through standard cultural practices. In another study, Keid *et al.* (2010) compared the sensitivity of PCR by using two different samples in 72 dogs. They compared both serum and blood PCR assays' sensitivity and concluded that blood PCR assay is more sensitive in detecting brucellosis infected dogs. Detection of brucellosis by PCR is not only applicable in dogs as Gupta *et al.* (2010) confirmed the results of their newly developed ELISA assay (for brucellosis detection) by using antibody gene specific PCR primers in goats.

Colibacillosis: It is a bacterial disease of animals that can also infect humans but it is more common in poultry birds (Rahman *et al.*, 2004). A review study by Kabir (2010) showed that avian colibacillosis is a major disease of chicken and its presence throughout the world could be the limiting factor for proliferation of chicken industry. Roy *et al.* (2009) found that 31.81% of calves were infected with colibacillosis and *E. coli* was present in all infected animals. In another study conducted in Spain on red-legged partridges (used for hunting), high prevalence rate of *E. coli* was reported (Diaz-Sanchez *et al.*, 2012). Prevalence of pathogen was higher in farm reared red-legged partridges (45%) as compared to wild and restocked red-legged partridges. It shows that persons working on farm are at high risk of developing this disease.

Dogs are susceptible to this disease especially in early days of puppies feeding. Infected genital tract is also a source of infection, which results in abortion or septicaemic (blood poisoning) death of puppies (Bjurstrom, 1993; Linde, 1983). In dogs the most common symptoms of this disease are dehydration, lack of appetite, increased heart rate, cold skin, watery diarrhea and secretion of bluish mucus membranes. A case of 90 days old puppy was observed, which died due to chronic diarrhea (Wada *et al.*, 1996). Further investigations showed the presence of *E. coli* attached with the stomach wall of infected puppy. Studies on *E. coli* (causative agent of colibacillosis) diagnosis by PCR have been reported in different organisms (Mora *et al.*, 2009; Seidavi *et al.*, 2010) but studies on PCR assay development to detect colibacillosis in dogs are scarce.

Giardiasis: It is a zoonotic disease more commonly reported in developing countries, especially in areas

where sanitation practices are very poor (Oberhuber *et al.*, 1997). *Giardia lamblia* is very common protozoan parasite of animals; a worldwide study was conducted to determine its prevalence (Feng and Xiao, 2011) and Uganda was found to be the place where its infection rate was highest among humans (40.7%). Julio *et al.* (2012) studied the *Giardia* infection rate among humans and found that its infection rate is higher in children of 0-5 years age, no difference in its prevalence was found between male and females. It was more prevalent in those who have contact with dogs and poor drainage system at homes. Varying infection rate has been reported in cattle (3.7-57.8%), sheep (1.5-55.6%), goats (12.3-42.2%), dogs (2-64.3%) and cats (2-44.4%) (Fayer *et al.*, 2006; Overgaauw *et al.*, 2009; Palmer *et al.*, 2008; O'Handly *et al.*, 2000; Ruiz *et al.*, 2008; Johnston *et al.*, 2010; Hsu *et al.*, 2007; Giangaspero *et al.*, 2005). The major source of its spread is through water bodies, contaminated with feces of infected animals (Marshall *et al.*, 1997). This disease transmits through ingesting infectious cysts from feces of infected animals and can be diagnosed by analyzing the fecal smear. Symptoms are acute, non-continuous or chronic in nature and in some cases soft, frothy and greasy diarrhea with a strong awful odor or mucus is reported.

In a study Paz-e-Silva *et al.* (2012) collected 300 fecal samples of dogs in Brazil; they analyzed these samples for detection of *Giardia duodenalis* through PCR. Small subunit ribosomal DNA (SSU-rDNA) and Glutamate Dehydrogenase (*GDH*) genes were targeted for PCR analysis and genetically characterized the *G. duodenalis* by Restriction Fragment Length Polymorphism (RFLP) and sequencing of *GDH* gene. Traub *et al.* (2009) conducted a study in Bangkok to analyze the prevalence of *G. duodenalis* in human and canine populations. They used *SSU-rDNA* gene for PCR detection and concluded that the PCR is the most reliable test for detection of *G. duodenalis*.

Lyme: Lyme is a bacterium disease mainly caused by *Borrelia burgdorferi*, a group of spirochete bacterial species transmitted by slow-feeding and hard-shelled deer ticks. It is a zoonotic disease discovered in Lyme town of USA in 1975. A study was conducted by Milewski *et al.* (2011) in USA to evaluate the Lyme arthritis in children, 31% children were found to be infected. Zhang *et al.* (2010) studied the prevalence of *Borrelia burgdorferi* sensu lato (causative agent of lyme) in rodents. They reported 22.86% infection rate in 7 species of rodents, these rodents serve as the source of this pathogen in environment. Seabirds may be the source of spreading

Lyme disease in environment and to check this hypothesis Duneau *et al.* (2008) studied a case of seabirds and found that 26% of seabirds were infected with Lyme borreliosis.

In dogs common symptoms of Lyme disease are difficulty in breathing, joint inflammation and difficulty in walking with arched back (Wasmoen *et al.*, 1992). Studying background history and blood testing of dog plays important role in diagnosis of this disease. Appel *et al.* (1993) used ELISA technique for its diagnosis and found high level of antibodies in infected dogs after 4-6 weeks of infection. In a case study Levy and Magnarelli, 1992) reported that only 5% of infected dogs showed physical signs of Lyme disease; it indicates that detection of this disease depending solely on its physical symptoms is not reliable.

Lyme disease is thought to be causing the neurological disorders and to confirm this Krimer *et al.* (2011) conduct a study on dogs that were experimentally challenged with *B. burgdorferi*-infected ticks. No DNA was found in tissues of dogs that were infected ticks carrying *B. burgdorferi*. Many other studies also reported poor detection of this disease by using PCR (Chou *et al.*, 2006; Hutton *et al.*, 2008; Leschnik *et al.*, 2010), on the other hand analysis of infected animals' seroreactivity for its diagnosis could serve the purpose very well (Kiss *et al.*, 2011; Mircean *et al.*, 2012). PCR could be useful to identify dogs that are at risk to this pathogen (*B. burgdorferi*) by isolating ticks from their bodies and then screening them through PCR, targeting the *B. burgdorferi* DNA (Smith *et al.*, 2012).

Leptospirosis: It is a bacterial infection caused by *Leptospira interrogans*, penetrating through skin and then spread throughout the body (Andre *et al.*, 1994). It occurs mostly under wet environmental conditions as these bacteria are more persistent in marshy or muddy areas. Contaminated water, mud and urine obtained from infected animals act as the source of infection. Jansen *et al.* (2007) studied the infection rate of leptospirosis in wild boars (18%) and found that wild boars are important source to cause infection in humans. Zhang *et al.* (2012) determined the spread of this zoonotic disease among humans during last two decades in China; its infection rate was high due to heavy rains and floods, out of total infected cases 60% humans were infected due to *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Lai. Prevalence of leptospirosis in patients suspected to be infected with dengue virus was observed by Sergio *et al.* (2012), they reported 48, 6 and 12% of suspected patients were infected with dengue, leptospirosis and both dengue and leptospirosis pathogens, respectively. About 34% of these suspected

individuals were negative to both infections. Prevalence of leptospirosis was high in patients suffering with dengue fever.

In dogs most commonly observed signs are sudden illness, restriction in muscle movement, shivering, weakness, lack of appetite, vomiting, spontaneous cough, yellow skin and diarrhea. Blood and urine analysis with whole history of symptoms are required for its better diagnoses. Due to zoonotic nature of this disease it should be handle very carefully (McDonough, 2001). Microscopic Agglutination Test (MAT) is the commonly used diagnostic method for leptospirosis detection. Miller *et al.* (2011) conducted a study in, which they compared the MAT results of same samples obtained from different laboratories. They concluded that though this test is good in diagnosing this disease but it is not valid in predicting the infecting serigroup. In addition to this, detection of pathogen at early stage of disease is not possible with MAT. Pathogens that cause Leptospirosis mostly colonize renal tubules of infected animals so analyzing the urine samples of infected animals could be helpful in detection of this disease. Rojas *et al.* (2010) designed a Real-Time PCR assay targeting the *lipL32* gene sequence, which is highly conserved among pathogenic leptospire. They analyzed 525 urine samples of dogs through real-time PCR and concluded that this disease was 7.05% prevalent among individuals of tested population. Detection of pathogen through real-time PCR is very fast as compared to antibody based diagnostic techniques (Slack *et al.*, 2007; Ahmed *et al.*, 2009).

Salmonellosis: It is an infection caused by *Salmonella* and host of this bacterium may carry more than one species of this bacterium, which serves as a source of its transmission. Readel *et al.* (2010) conducted a study to observe turtles as carrier and source of salmonellosis infection, *Salmonella* was found in 11% of total population under study, it clearly shows the high prevalence rate of this pathogen in turtles. Prevalence of *Salmonella* in shedding of cattle herds was observed (Cummings *et al.*, 2010) and 77% of herds indicated the prevalence of *Salmonella*. A survey for *Salmonella* prevalence was carried out at a pig farm in Spain by Vico *et al.* (2011) and they found *Salmonella* was present in 94% of studied herds; moreover, 31% of studied individuals were infected with this pathogen. These animals can be the source of disease transmission in humans, Guo *et al.* (2011) estimated the relative contribution of food products to cause infection in humans by using a model developed by them. They estimated the relative contribution of chicken (48%) ground beef (28%) turkey (17%) and egg (6%) products in causing infection to humans.

Young and old dogs are more susceptible to it because of low immunity levels. Among its symptoms fever, diarrhea, vomiting, anorexia, weight loss, dehydration, vaginal discharges and swollen lymph nodes are most common. It is difficult to diagnose because its symptoms overlap with symptoms of other diseases, so urine and feces samples are analyzed in laboratory for its diagnosis. Finley *et al.* (2007) conducted an experiment in, which they fed *Salmonella* contaminated diet to 12 dogs. They found *Salmonella* in feces of seven dogs and concluded that under natural conditions feces of infected dogs could serve as source for spread of this disease. Moreover they didn't find any clinical signs of infection in infected dogs.

Commonly used methods for the detection of pathogen causing salmonellosis are selective culture methods, biochemical identification and serotyping; all these methods are time consuming and laborious in nature. Real-time PCR is fast and specific for pathogen detection, hence it could play a useful role to overcome the demerits of conventionally used diagnostic methods for detection of this pathogen (Marks *et al.*, 2003). In another study Schuurman *et al.* (2007) conducted a research and compared different diagnostic methods with real-time PCR to detect *Salmonella enterica*. They concluded that by employing real-time PCR, on an average 2.9-3.8 days can be saved as compared to conventional diagnostic methods.

Campylobacteriosis: It is a bacterial infection caused by *Campylobacter jejuni* or *C. coli*, which lives in gut (gastrointestinal tract) of healthy mammals and food serves as a source of its infection in humans. A study was conducted to observe the prevalence of *Campylobacter* in different food items in Pakistan (Hussain *et al.*, 2007), overall prevalence of *Campylobacter* was 21.5% out of, which prevalence rate of *C. jejuni* and *C. coli* was 70.6 and 29.6%, respectively. In addition, they reported the varying prevalence rate of this pathogen in different food items like chicken (48%), beef (10.9%), mutton (5.1%), vegetable salad (40.9%), sandwiches (32%), cheese (11%) and milk (10.2%). Another study was conducted to determine the prevalence of *Campylobacter* sp. (Strachana *et al.*, 2012) in liver of different animals obtained from retail market; pathogen was detected in 81, 69, 79 and 78% of chicken, cattle, pig and sheep samples, respectively. Moreover, it was found that strain of pathogen isolated from chicken liver was similar to human infecting strain. Madoroba *et al.* (2011) studied the prevalence of *Campylobacter* among Southern African cattle and reported 1.9% prevalence of *C. foetus*, which is a causative agent of infertility and abortion in these animals.

It is more common disease in puppies with age of <6 six months; it can be transmitted to non-infected animals through contaminated feces and food. The most common signs are vomiting, anorexia, fever and enlarged lymph nodes. A study was conducted to observe the transmission of *Campylobacter jejuni* or *C. coli* in dogs, chicken and humans (Workman *et al.*, 2005). *Campylobacter jejuni* was also detected in humans (63.6%), chickens (86.6%), dogs (51.5%) and chicken meat (79.8%); in addition to this chicken meat was found to be the main source of its transmission to humans.

Detection of this pathogen by using DNA based diagnostic techniques has been reported by many researchers, Chaban *et al.* (2009) developed a real-time PCR assay using *cpn60* gene specific primers for detection of different *Campylobacter* species. After optimization of real-time PCR assay they analyzed fecal samples of dogs obtained from dogs and concluded that this newly developed assay will facilitate the investigators to study *Campylobacter* species without employing any bacterial culture technique. Many other researchers have used real-time PCR assay to detect *Campylobacter* species by using *cpn60* gene specific primers (Chaban *et al.*, 2010, 2012).

Urinary tract infections: Some bacteria may enter and colonize in bladder or upper portion of urethra resulting in infection, which is commonly known as Urinary Tract Infection (UTI). Kolawole *et al.* (2009) studied the prevalence of UTI among patients, examined at Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria, 60% of patients were infected. Another case of ill children was studied by O'Brien *et al.* (2011), they reported 4% urinary tract infection among studied children population. In another study, Shaikh *et al.* (2008) found that prevalence of UTI was 7% in studied population of children. The persons with weak immune system due to any other disease are at high risk of developing this disease.

Dogs of all age are at risk of this infection but older and female dogs are more susceptible to these infections. *E. coli*, *Staphylococcus* and *Proteus* sp. are the commonly found causative agents of this infection. The most commonly observed signs are difficulty in urinating, blood in urine and continuous urination in small amounts. Urine analysis is more valuable for its diagnosis; presence of pus and blood are among the initial signs of infection. To identify pathogen responsible for UTI, 385 samples obtained from dogs showing disease symptoms were analyzed (Wooley and Blue, 1976). Researchers identified

three bacterial types; *Escherichia coli*, *Proteus* sp. and *Staphylococcus aureus* among all tested samples. Olby *et al.* (2010) reported that chances of developing UTI are high in dogs with acute intervertebral disc extrusion. Available data on diagnosis of UTI in dogs is not enough to develop a PCR assay with reproducible results. But the common genetic background of pathogenic *E. coli* strains of animals and humans (Clermont *et al.*, 2011) could be used to develop new PCR assays on the basis of previous reports on detection of *E. coli* in different organisms.

CONCLUSION

Early detection of infection is essential to ensure effective control of disease as the time of infection increases so do the microbial load in body; infection can be controlled easily when pathogens are less in number. Moreover, chances of resistance development against used antimicrobial increases with the number of pathogens. The need of early infection control becomes more intense when we deal with pathogens that are of zoonotic nature to ensure the health of both humans and animals. Dog is one of the most common domestic animals and often develops a close physical relationship with humans. Hence, current review was conducted to through some light on prevalence and diagnosis of dog bacterial diseases that are also transmittable to humans. PCR was found to be effective in detection of pathogen causing brucellosis, giardiasis, leptospirosis, salmonellosis and campylobacteriosis but when dealing with Lyme, PCR doesn't seem to be much effective. In case of colibacillosis and UTI sufficient data was not available for detection of these diseases through PCR, which emphasizes the need of future research on developing PCR assays for detection of these diseases at their early stage of development. Knowledge about conserved genomic regions and genes that are important for virulence of these pathogens will be helpful to develop organism independent PCR assays. Many other DNA based diagnostic techniques (e.g., RFLP) can be combined with PCR to increase the accuracy of pathogen detection. For example *E. coli* is a commonly found organism in microflora of both humans and animals, some of its strains are pathogenic in nature so combination of PCR with other DNA based diagnostic techniques could be used for precise detection of pathogenic bacterial strains. In nutshell PCR is a good technique for early detection of pathogenic bacteria but still further research is required to cover all bacterial diseases.

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