Journal of Animal and Veterinary Advances 11 (15): 2804-2812, 2012
ISSN: 1680-5593

PCR Based Diagnosis of Fungal Diseases in Dogs

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Abstract: Interaction with animals provide necessary companionship and helps people to live better life by reducing risk of many health problems, improved fitness and act as a source of social enjoyment. In modern society there is a substantial increase in the number of dogs adopted as pet which also raises the concerns regarding the transmission of infections from dog to their owners and vice versa. Early diagnosis of infected dogs could prevent the owners from these infections. In last decade, PCR has proven its potential applications as an important diagnostic tool but these applications are mainly limited to the diagnosis of human diseases and very little work has been done on animals. Development of PCR assays for detection of commonly found canine fungal infections (aspergillosis, blastomycosis, coccidioidomycosis and cryptococcosis) is of utmost importance in order to ensure the early and accurate diagnosis of infection. Although, a lot of research is being done on molecular diagnosis of animal infectious disease but most of these newly developed assays are either not well optimized or only suitable for laboratory studies. On the basis of reviewed literature it can be concluded that more research work is required to develop an efficient (rapid, sensitive and cost effective) PCR assays for the diagnosis of canine fungal infections.

Key words: aspergillosis, blastomycosis, coccidioidomycosis, cryptococcosis, BAD gene, CAP59

INTRODUCTION

These days the adaptation of pets is considered as healthy activity because they are often found to have link with induction of well being in people and development of social skills in children. The level of their significance in homes can also be judged by the fact that about 62% of US household have a pet (APPA, 2011). Although, pets have various beneficial social effects on our life but they are also considered as a source of zoonotic diseases (Gavgani *et al*., 2007). They not only act as a source of direct transmission of diseases to humans but also contaminate their surrounding environment which later on indirectly transmits these diseases to human. Early detection of infected animals may reduce the risk of disease transmission to humans especially in immunocompromised patients.

In case of fungal infections, combination of microscopy and culture-based techniques are commonly used for detection purpose. The microscopic examination proves its significance as a rapid method of detection but is not considered good with respect to its sensitivity and specificity (Hayden *et al*., 2001). On the other hand culturing the microorganism provides accurate identification but requires time, resulting in delayed diagnosis and further initiation of appropriate therapy. In addition to this a considerable level of expertise and strict adhesion to laboratory safety rules are some prerequisites for practical application of these techniques. With the advancement in molecular biology tools and their potential applications in vast field of research (Moghim *et al*., 2007; Behbahani, 2012; Sohail *et al*., 2012) the task of rapid and accurate detection of infectious agents has become more easier. The use of fungal nucleic acid-based test into the clinical microbiology laboratory has significantly improved the diagnosis of fungal infections, especially the PCR (Espy *et al*., 2006). However, in most of the cases, a significant amount of data is lacking to support the standardization of these tests for effective laboratory diagnosis. The situation becomes worse when data based on samples obtained from domestic animals is under question. Therefore, the literature on diagnosis of some common fungal diseases of dog was reviewed in order to put some light on current scenario.

Aspergillosis: It is a fungal infection caused by *Aspergillus* spp., found everywhere as mold (spores); to

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date more than 100 species of *Aspergillus* have been identified. It may cause infection to humans as well as animals; most common infection causing species are *A. flavus and A. clavatus*. Aspergillosis is more common in people with compromised immune system. Mannose-Binding Lectin (MBL) deficiency is usually found in 20-30% of human population; a study was conducted to determine the role of MBL as a susceptibility factor for invasive aspergillosis in humans (Lambourne *et al*., 2009). The lower level of MBL was observed in aspergillosis infected patients (281 ng mL^{-1}) as compare to control subjects (835 ng mL^{-1}), supporting the positive association of MBL deficiency with aspergillosis infection. Presence of zoonotic agents was observed in dogs like *Leptospira interrogans*, *Trypanosoma cruzi* and *Aspergillus* spp., (Jimenez-Coello *et al*., 2010). *A. niger* (82.8%), *A. flavus* (14.3%) and *A. terreus* (2.9%) were identified out of 35.7% dogs that contained *Aspergillus* spp. They concluded that dogs are an important source of infection transmission to humans.

Its prevalence is worldwide in animals as well as humans; a study was conducted to observe the prevalence of Allergic Bronchopulmonary Aspergillosis (ABPA) and sensitivity to *Aspergillus* antigens in asthmatic patients (Ma *et al*., 2011). A total of 5.5% patients showed skin reactivity to *Aspergillus* antigens and 45.5% of these were found to have ABPA. A case was studied to determine the prevalence of ABPA and *Aspergillus* Hypersensitivity (AH) in asthma patients (Agarwal *et al*., 2009). Prevalence of AH and ABPA was found to be 28 and 12.9%, respectively; moreover, researchers concluded that patients of bronchial asthma are even more susceptible to AH and ABPA infection. Flynn and Weil (2009) studied the temporal and spatial variable prevalence of aspergillosis in *Gorgonia ventalina* present in La Parguera, southwest Puerto Rico. They reported an increase in infection rate from 5.1±9.5% (2005) to 14.4±16.0% (2006). This disease was more prevalent in shallow areas because of standing water and high temperature. Among domestic animals dogs and cats are highly susceptible to this disease and mainly gets two types of infection; nasal and disseminated. *A. fumigatus* mostly found in respiratory tract of birds; hence pet birds are at high risk of developing this infection.

In dogs aspergillosis transmits through contact with mould contaminated dust, hay and grass; nasal pain, bleeding from nose, visible swollen nose, nasal discharge having mucus or pus and loss of appetite are common signs for nasal type of infection. Contrary to this in disseminated type of infection, inflammation of animal's bone and spinal pain is mostly observed. The sinonasal or nasal type of infection is most common in dogs and it is diagnosed by observing the physical symptoms like nasal discharge and other related clinical symptoms. During endoscopy or histopathologic examination, direct visualization of fungal plaques is most commonly used methods for its diagnosis (Peeters and Clercx, 2007). After visual diagnosis further confirmation is done by serology but recent studies have raised some concerns regarding its sensitivity (Mennink-Kersten *et al*., 2004; Pomrantz *et al*., 2007). The detection of pathogenic DNA could facilitate the early disease detection and if used in combination with serology the problem of sensitivity can be resolved. Adamama-Moraitou *et al*. (2011) studied the case of a three year old female Hellenic shepherd dog and identified the *Aspergillus fumigates* after sequencing the PCR product of internal transcribed spacer regions 1 and 2 from genetic material which was extracted from the pulmonary tissues.

The concentration of fungal DNA present in samples is highly important for successful diagnosis of disease through PCR assay. Peeters *et al*. (2008) conducted a study in which they compared the performance of real-time PCR assays in detecting the DNA of *Aspergillus* among different samples obtained from infected dogs. They analyzed the blood and nasal tissue samples of diseased individuals and finally concluded that whole blood samples doesn't play significant role in detecting the fungal DNA. In another study based on human samples, White *et al*. (2011a) evaluate the sensitivity of PCR test for *Aspergillus* detection in 23 different European centers. They found positive association of PCR sensitivity with internal PCR control, large sample volume and internal transcribed spacer as target region of PCR; however, sensitivity was found to be inversely correlated with PCR accuracy in which mitochondrial genes were used as target region. There are many factors like concentration of DNA in sample, selection of target gene and internal control that hinders the development of PCR as a diagnostic tool for *Aspergillus*. Recently White *et al*. (2011b) conducted a study in which they compared the performance of a commercially available Myconostica MycAssay *Aspergillus* PCR (MAP) assay with validated In-house *Aspergillus* PCR (IHP). They found the results of MAP to be comparable with IHP and other commercially available tests; however, they emphasized on the need of evaluating the MAP assay at larger scale in order to improve its clinical evaluation. Though the process of PCR optimization for *Aspergillus* detection in human beings is in its final stage but still efforts are required to develop a PCR based diagnostic assay to detect *Aspergillus* in canines.

Blastomycosis: It is a fungal disease of humans as well as animals caused by *Blastomyces dermatitidis* which is mostly found in moist soils associated with decomposed organic matter. It may cause infection after inhaling its airborne microscopic spores from the environment and its symptoms are similar to flu disease due to which it is hard to diagnose (Lupi *et al*., 2005). Some of infected persons show severe pulmonary infection but majority is either asymptomatic or show some respiratory symptoms (Smith and Kauffman, 2010). It is a zoonotic disease as its transmission from different animals to humans has also been reported by some researchers (Harris *et al*., 2011).

Its prevalence is worldwide among humans and animals; it is endemic especially, in border areas of Mississippi and Ohio rivers and South Central and Midwestern states in America (McKinnell and Pappas, 2009). A study was conducted to observe the prevalence of human blastomycosis as endemic disease in northeast mountains of America (Hussein *et al*., 2009). More than half of total cases of blastomycosis were reported during study period (1996-2005); researchers concluded that it persists as an endemic fungal infection in the core area of two counties: Washington and Unicoi. Herrmann *et al*. (2011) studied a case to compare the temporal and spatial distribution of blastomycosis in dogs and humans. They reported increased frequency of blastomycosis among humans from 3.8-10.7 cases/million person/year during study period (2001-2007) while in dogs it was 8.3 times more common than humans.

Male dogs are more susceptible to blastomycosis as compare to female dogs and get infection after inhaling airborne fungal spores of *B. dermatitidis*; they may get infection by getting in contact with contaminated soil or decaying wood (Kerl, 2003). Most commonly observed symptoms are fever, weight loss, eye discharge and inflammation, skin lesions and difficulty in breathing. It has clinical symptoms which are similar to many other diseases, i.e., hind-limb lameness; in a study Oshin *et al*. (2009) confused lytic lesions of blastomycosis with chronic left hind-limb lameness in a dog, but after biopsy they confirmed it as blastomycosis. Physical, cytological and radiographic means are commonly adopted to examine the infected tissues for disease diagnosis (Garma-Avina, 1995; McMillan and Taylor, 2008; Crews *et al*., 2008). Its early diagnosis is very important for effective disease control; in this regard serologic testing plays an efficient role but there are some reports in which these serologic tests often results negative at early disease stage (Bromel and Sykes, 2005; Dial, 2007). To resolve this problem PCR could be used as powerful tool for early and effective disease diagnosis. Bialek *et al*. (2003) conducted a study to detect the *B. dermatitidis* in paraffin-embedded canine tissue by targeting the gene encoding *B. dermatitidis* adhesion (*BAD*) formerly known as *Wl-1*, in a nested PCR assays. The PCR assay successfully identified the *B. dermatitidis* but its detection was limited to those samples in which organism was also detected through histology. Babady *et al*. (2011) developed a real-time PCR assay to detect *B. dermatitidis* in clinical samples and found the specificity and sensitivity of this assay 100%. Recently Sidamonidze *et al*. (2012) developed a real-time PCR assay to detect *B. dermatitidis* in culture and clinical samples by targeting its *BAD1* gene promoter. They found this newly developed assay very efficient as it can identify the infected specimens within five hours. Many reports support the effectiveness of PCR in detecting the *B. dermatitidis* in human samples but studies focusing the samples obtained from dogs are still rare. Further research is required in future specifically focusing the optimization of PCR assays for *B. dermatitidis* detection in infected dogs.

Coccidioidomycosis: It was first reported from Argentina in 1890, primarily coccidia (protozoa) like pathogens were found during the biopsies but later on (1896-1900) further investigations revealed it as fungus. This fungal disease is commonly known as valley fever caused by *C. immitis* or *C. posadasii* and endemic to many southwestern parts of America (Chiller *et al*., 2003). It remains dormant during dry season and develops molds in rainy season which break off into air borne spores (arthroconidia). Infection is caused by inhalation of these particles; infected organism usually develops immunity against this fungus after its first infection.

Coccidioidomycosis is one of the oldest mycosis; an increase in coccidioidomycosis infection was reported in California and Arizona due to massive migration from normal to endemic areas (Laniado-Laborin *et al*., 2012). This disease is endemic in regions of western hemisphere between 40° latitudes north and south. In Arizona, increase in infection rate was reported by Park *et al*. (2005), who conducted a study to determine the reasons for increased spread of this disease. They found an increase in number of reported cases and relate this increase with climate change. In another study Ampel (2010) reported an increase in number of cases in the endemic regions of California and Arizona during the last two decades, reports of San Joaquin valley showed a huge increase >150 per 100,000 of population. They also correlated this increase with climate changes and other factors like local exposure and increased susceptibility of people to this infection. Some studies also showed the high infection rate of this disease in males (Cordeiro *et al*., 2009). The investigators further described that most of the infected males were between the 18-65 years of age.

This fungus can also infect animals; a pulmonary infection was reported in a dog and cat that further developed into chronic cough (Graupmann-Kuzma *et al*., 2008). Serological tests are supportive in diagnosis but its diagnosis is based on *Coccidioides* identification in cytological or tissue samples. In dogs, it is caused by inhalation of soil-borne fungus which normally attacks the dog's respiratory system. The most common symptoms are fever, lameness, coughing, difficulty in breathing, skin ulcers, inflammation of cornea and weight loss. Kirsch *et al*. (2012) researched on the detection of *Coccidioides* antigen in dogs; they reported 3.5, 19 and 20% antigen detection from samples of urine, serum and mixture of urine and serum, respectively. Bialek *et al*. (2004) conducted a study in which they developed the conventional nested PCR and a real-time LightCycler PCR assay for the detection of *C. posadasii*. They isolated 120 clinical strains of *C. posadasii* from 114 patients and target the gene encoding for a specific antigen 2/proline-rich antigen (Ag2/PRA) for its DNA detection in all clinical samples. Both of the developed assays successfully identified the 120 clinical isolates as *C. posadasii* positive. In another study De Aguiar Cordeiro *et al*. (2007) performed the same PCR assays with little modifications and find similar results; in this modified method alternate DNA extraction method was adopted as it is limited to the Biosafety Level 3 laboratories. To compare the performance of PCR with other typically used diagnostic tests Vucicevic *et al*. (2010) conducted a study in which they analyzed the data obtained from 145 patients who underwent *Coccidiodes* PCR during the course of 2007-2008. They finally concluded that *Coccidiodes* PCR was accurate in identifying the negative results and its sensitivity is comparable to that of fungal culture. Many other studies have also proven the significance of PCR assay in diagnosing the coccidioidomycosis but their focus remains limited to human samples only (Binnicker *et al*., 2007; Kishi *et al*., 2008). Studies conducted on human samples can be helpful in developing the PCR assays for detection of coccidioidomycosis in dogs.

Cryptococcosis: It is a fungal disease caused by *Cryptococcus* genus; *Cryptococcus neoformans* and *Cryptococcus gattii* are its most commonly found species which may infect both humans and animals (Springer and Chaturvedi, 2010; Sidrim *et al*., 2010). It was termed as "Busse-Buschke disease" earlier, after the name of two individuals who identified the causative fungus for the very first time in 1884-1895. It is more common among patients with weak immune system, i.e., HIV patients; it spreads through spores and bird's feces; *C. gattii* is associated with airborne spores while *C. neoformans* is found in most bird's feces, especially in old pigeons and bat guano. Birds are not susceptible to this infection; but they can act as its carrier to infect humans and other animals. In humans, most common symptoms are fever, dry cough, chest pain, headache, blurred vision, confusion and fatigue.

Its prevalence is worldwide; Park *et al*. (2009) reported that at least 500,000 deaths occur annually worldwide due to HIV associated with cryptococcosis. A case of 573 HIV seropositive patients was studied in India by Baradkar *et al*. (2009) to observe the prevalence of *C. meningitis;* they reported 2.79% prevalence accompanied with symptoms like fever, headache, altered sensorium and neck stiffness. Another study was conducted by Thakur *et al*., (2008) to determine the prevalence of CNS (central nervous system) cryptococcosis in patients suspected for HIV infection. They found that 12.5% of patients from their study group were infected with HIV and out of these 46% were positive for CNS cryptococcosis. Many other studies also support this hypothesis that his disease is more common in patients with compromised immune system (Oyella *et al*., 2012).

Cryptococcosis is a common fungal disease among cats but often found in dogs which usually leads to the chronic infection of nose and sinuses (Malik *et al*., 1995). It is more common in those animals which live near the site of commercial environmental disturbance (Duncan *et al*., 2006). It spreads systematically in dogs; fungus is contracted through nasal passage then leads to brain, eyes, lungs and other tissues. Most common symptoms of this disease in dogs are nervous system disorders, enlarged lymph nodes, vomiting, diarrhea, lack of appetite and nasal discharge depending upon the affected organ system. It can be diagnosed by various methods like biopsy, examining the spinal cells, blood and urine culture for detection of cryptococcosis antigens. For rapid detection of this pathogen (Cordeiro *et al*., 2011) developed a PCR-REA method and analyzed samples collected from various human and veterinary sources. They targeted the capsular region of *Cryptococcus* for PCR amplification and then treated the PCR product with restriction enzymes. They found this newly developed method faster and more sensitive than previously used methods as it can also detect *C. gattii* samples. The gene *CAP59* of both *C. neoformans* and *C. gattii* has also been successfully used in a real-time assay for detection and identification of these two fungal pathogens (Satoh *et al*., 2011). Although, a considerable number of studies have been published recently (Veron *et al*., 2009; Favaro *et al*., 2012), supporting the productive role of PCR

in the diagnosis of cryptococcosis but spectrum of these studies is only limited to human samples and in future research is required to develop DNA based methods for cryptococcosis diagnosis in dogs.

CONCLUSIONS

Daily interaction with animals do has some positive medical effects on our lives i.e., low blood pressure, reduction in both cholesterol levels and heart related health problems and many more. However, if these animals are infected with any pathogenic organism which is transferable to humans then their interaction could lead towards the development of severe infectious disease especially in immunocompromised individuals. Advancement in medical field has lead to the better health conditions in general population; organ and stem cell transplantation are among the promising contributing factors that are responsible for improved clinical practices in medical science. But introduction of immunosuppressive therapies, especially in those patients who undergo organ or stem cell transplantation put them at high risk of getting fungal infections (Cuenca-Estrella *et al*., 2008). Under such scenario use of antifungal compounds is inevitable but recent research findings indicate the development of resistance in fungal pathogen against used antifungal compounds (Pfaller *et al*., 2011; Barlian *et al*., 2011). This situation is not limited to fungal pathogens, as there are studies reporting the emergence of antibiotic resistant strains of various bacterial pathogens (Farrag, 2001; Khanum *et al*., 2006; Yousefi-Mashouf and Hashemi, 2006; Adeniyi *et al*., 2006; Luga *et al*., 2007; Abongo *et al*., 2008; Patel *et al*., 2012; Kumar *et al*., 2012; Geidam *et al*., 2012). Although, new synthetic antimicrobial compounds are being reported continuously but the pace of discovering new compounds is slower as compared to emergence of resistant strains. This reason forced the scientists to look for alternate sources to discover new antimicrobial compounds (Ismail *et al*., 2003; Asha Devi *et al*., 2006; Malabadi and Kumar, 2007; Zongo *et al*., 2010; Momtaz and Abdollahi, 2010; Sarwar *et al*., 2011; Sohail *et al*., 2011; Sohail and Sohail, 2011; Sumathi *et al*., 2012). But still the situation is quite severe and strategies are required to deal with these infectious agents other than the antimicrobial compounds.

Many fungal pathogens that infect both humans and dogs are common in immunocompromised individuals and flourish in moist environments e.g., causal agents of aspergillosis, blastomycosis, coccidioidomycosis and cryptococcosis. Prevention of immunocompromised patients from various opportunistic moulds is one of the few practicable options. Although, control of these infection-causing agents in environment is difficult but avoidance of infection source could be an effective way to save the patients from getting fungal infections. The screening of animals having fungal infection at early stage will be an effective strategy to isolate them from healthy but immunocompromised persons to limit the spread of infection in human population. Techniques like microscopy, serology and organism culture have been used as the main diagnostic tools for fungal infections for such a long time but delay in diagnosis, lack of sensitivity or specificity are some problems associated with them. Moreover detection of infection at its early stage is also difficult by employing these techniques. Introduction of molecular techniques and especially the PCR have made possible the detection of infection at early stage of its development. However, this technique has not been well optimized as a diagnostic tool for most of the animal infections, but study of optimized PCR assays for detection of human infections will be very supportive for animal studies. Sample type and target sequence of pathogenic DNA are two important factors which needs to be optimized for the development of an efficient diagnostic PCR assay. The effective type of sample is usually the one that is collected from the site of infection, as in case of *Aspergillus* nasal samples provide more efficient detection of pathogen than blood samples (Peeters *et al*., 2008). On the other hand selection of target gene is also very crucial and *BAD* (blastomycosis) and *CAP59* (cryptococcosis) genes of different pathogens have been successfully used in different nested and real time PCR assays to detect the pathogens that are responsible for these infections (Bialek *et al*. (2003; Cordeiro *et al*., 2011). Though many studies are available, aiming to optimize the various types for PCR but those mainly target the human samples and more efforts are required to establish/optimize new PCR assays for early detection of these diseases in domestic animals.

REFERENCES

APPA, 2011. APPA national pet owners survey 2011-2012. American Pet Products Association, Washington D.C., USA. http://www.americanpetproducts.org/pubs_survey.asp

Abongo, B.O., M.N.B. Momba, V.K. Malakate and J.N. Mwambakana, 2008. Prevalence of *Escherichia coli* O157:H7 among Diarrhoeic HIV/AIDS patients in the Eastern Cape Province-South Africa. Pak. J. Biol. Sci., 11: 1066-1075.

Adamama-Moraitou, K.K., D. Pardali, M.J. Day, D.W. Denning, L. Papazoglou, A. Papastefanou and T.S. Rallis, 2011. *Aspergillus fumigatus* Bronchopneumonia in a Hellenic shepherd dog. J. Am. Anim. Hosp. Assoc., 47: e13-e18.

Adeniyi, B.A., F.A. Ayeni and S.T. Ogunbanwo, 2006. Antagonistic activities of lactic acid bacteria isolated from Nigerian fermented dairy food against organisms implicated in urinary tract infection. Biotechnology, 5: 183-188.

Agarwal, R., A.N. Aggarwal, D. Gupta and S.K. Jindal, 2009. *Aspergillus* hypersensitivity and allergic bronchopulmonary aspergillosis in patients with bronchial asthma: Systematic review and meta-analysis. J. Tuberc. Lung Dis. 13: 936-944.

Ampel, N.M., 2010. What's behind the increasing rates of Coccidioidomycosis in Arizona and California? Curr. Infect. Dis. Rep., 12: 211-216.

Asha Devi, N.K., M. Jeyarani and K. Balakrishnan, 2006. Isolation and identification of marine actinomycetes and their potential in antimicrobial activity. Pak. J. Biol. Sci., 9: 470-472.

Babady, N.E., S.P. Buckwalter, L. Hall, K.M.L. Febre, M.J. Binnicker and N.L. Wengenack, 2011. Detection of *Blastomyces Dermatitidis* and *Histoplasma capsulatum* from culture isolates and clinical specimens by use of Real-Time PCR. J. Clin. Microbiol., 49: 3204-3208.

Baradkar, V., M. Mathur, A. De, S. Kumar and M. Rathi, 2009. Prevalence and clinical presentation of *Cryptococcal* meningitis among HIV seropositive patients. Indian J. Sex Transm. Dis., 30: 19-22.

Barlian, A., K. Anggadiredja and A. Kusumorini, 2011. Damage in fungal morphology underlies the antifungal effect of lyophilisate of granular gland secretion from *Duttaphrynus melanostictus* frog. J. Biol. Sci., 11: 282-287.

Behbahani, A., 2012. Wolbachia infection and mitochondrial DNA comparisons among Culex mosquitoes in South West Iran. Pak. J. Biol. Sci., 15: 54-57.

Bialek, R., A.C. Cirera, T. Herrmann, C. Aepinus, V.I. Shearn-Bochsler and A.M. Legendre, 2003. Nested PCR assays for detection of *Blastomyces dermatitidis* DNA in paraffin-embedded canine tissue. J. Clin. Microbiol., 41: 205-208.

Bialek, R., J. Kern, T. Herrmann, R. Tijerina, L. Cece'as, U. Reischl and G.M. Gonzalez, 2004. PCR assays for identification of *Coccidioides posadasii* based on the nucleotide sequence of the antigen 2/proline-rich antigen. J. Clin. Microbiol., 42: 778-783.

Binnicker, M.J., S.P. Buckwalter, J.J. Eisberner, R.A. Stewart, A.E. McCullough, S.L. Wohlfiel and N.L. Wengenack, 2007. Detection of *Coccidioides* species in clinical specimens by real-time PCR. J. Clin. Microbiol., 45: 173-178.

Bromel, C. and J.E. Sykes, 2005. Epidemiology, diagnosis and treatment of blastomycosis in dogs and cats. Clin. Tech. Small Anim. Pract., 20: 233-239.

Chiller, T.M., J.N. Galgiani and D.A. Stevens, 2003. Coccidioidomycosis. Infect. Dis. Clin. North Am., 17: 41-57.

Cordeiro, R.A, A.K. Costa, R.S. Brilhante, R.A.C. de Lima and D.S.C.M. Castelo-Branco *et al*., 2011. PCR-REA as an important tool for the identification of *Cryptococcus neoformans* and *Cryptococcus gattii* from human and veterinary sources. Vet. Microbiol., 154: 180-184.

Cordeiro, R.A., M.A.B. Fechine, R.S.N. Brilhante, M.F.G. Rocha and A.K.F. da Costa *et al*., 2009. Serologic detection of coccidioidomycosis antibodies in Northeast Brazil. Mycopathologia, 167: 187-190.

Crews, L.J., D.A. Feeney, C.R. Jessen and A.B. Newman, 2008. Radiographic findings in dogs with pulmonary blastomycosis: 125 cases (1989-2006). J. Am. Vet. Med. Assoc., 232: 215-221.

Cuenca-Estrella, M., L. Bernal-Martinez, M.J. Buitrago, M.V. Castelli, A. Gomez-Lopez, O. Zaragoza and J.L. Rodriguez-Tudela, 2008. Update on the epidemiology and diagnosis of invasive fungal infection. Int. J. Antimicrob. Agents, 32: S143-S147.

De Aguiar Cordeiro, R., R.S. Nogueira Brilhante, M.F. Gadelha Rocha, F.E. Araujo Moura, Z. Pires de Camargo and J.J. Costa Sidrim, 2007. Rapid diagnosis of coccidioidomycosis by nested PCR assay of sputum. Clin. Microbiol. Infect., 13: 449-451.

Dial, S.M., 2007. Fungal diagnostics: Current techniques and future trends. Vet. Clin. North Am. Small Anim. Pract., 37: 373-392.

Duncan, C.G., C. Stephen and J. Campbell, 2006. Evaluation of risk factors for *Cryptococcus gattii* infection in dogs and cats. J. Am. Vet. Med. Assoc., 228: 377-382.

Espy, M.J., J.R. Uhl, L.M. Sloan, S.P. Buckwalter and M.F. Jones *et al*., 2006. Real-Time PCR in clinical microbiology: Applications for routine laboratory testing. Clin. Microbiol. Rev., vol 19, 10.1128/CMR.00022-06

Farrag, H.A., 2001. Post irradiation effect on adherent growth, slime formation and antibiotic resistance of *Pseudomonas aeruginosa* causing human infection. J. Med. Sci., 1: 244-250.

Favaro, M., V. Savini, C. Favalli and C. Fontana, 2012. A multi-target real-time PCR assay for rapid identification of meningitis-associated microorganisms. Mol. Biotechnol., 10.1007/s12033-012-9534-7

Flynn, K. and E. Weil, 2009. Variability of aspergillosis in Gorgonia ventalina in La Parguera, Puerto Rico. Caribbean J. Sci., 45: 215-220.

Garma-Avina, A., 1995. Cytologic findings in 43 cases of blastomycosis diagnosed ante-mortem in naturally-infected dogs. Mycopathologia, 131: 87-91.

Gavgani, A.S.M., A. Ghazanchaei, P. Karimi, H. Mohit and C.R. Davies, 2007. Practical approach for typing strains of *Leishmania infantum* by enzyme polymorphism: A cross sectional study in Northwest of Iran. Pak. J. Biol. Sci., 10: 4505-4509.

Geidam, Y.A., Z. Zakaria, S.A. Aziz, S.K. Bejo, J. Abu and S. Omar, 2012. High prevalence of multi-drug resistant bacteria in selected poultry farms in Selangor, Malaysia. Asian J. Anim. Vet. Adv., 7: 891-897.

Graupmann-Kuzma, A., B.A. Valentine, L.F. Shubitz, S.M. Dial, B. Watrous and S.J. Tornquist, 2008. Coccidioidomycosis in dogs and cats: A review. J. Am. Anim. Hosp. Assoc., 44: 226-235.

Harris, J.R., D.D. Blaney, M.D. Lindsley, S.R. Zaki and C.D. Paddock *et al*., 2011. Blastomycosis in man after kinkajou bite. Emerg. Infect. Dis., 17: 268-270.

Hayden, R.T., X. Qian, G.D. Roberts and R.V. Lloyd, 2001. *In situ* hybridization for the identification of yeastlike organisms in tissue section. Diagn. Mol. Pathol., 10: 15-23.

Herrmann, J.A., S.L. Kostiuk, M.S. Dworkin and Y.J. Johnson, 2011. Temporal and spatial distribution of blastomycosis cases among humans and dogs in Illinois (2001-2007). J. Am. Vet. Med. Assoc., 239: 335-343.

Hussein, R., S. Khan, F. Levy, J.B. Mehta and F.A. Sarubbi, 2009. Blastomycosis in the mountainous region of northeast Tennessee. Chest, 138: 1019-1023.

Ismail, M., Z. Iqbal, B. Ahmad, S. Zakir and U. Niaz, 2003. Biological and pharmacological properties of two indigenous medicinal plants, *Rheum emodi* and *Paeonia emodi*. Pak. J. Biol. Sci., 6: 984-986.

Jimenez-Coello, M., A. Ortega-Pacheco, E. Guzman-Marin, D.M. Guiris-Andrade, L. Martinez-Figueroa and K.Y. Acosta-Viana, 2010. Stray dogs as reservoirs of the zoonotic agents *Leptospira interrogans*, *Trypanosoma cruzi* and *Aspergillus* spp. in an urban area of Chiapas in southern Mexico. Vector Borne Zoonotic Dis., 10: 135-141.

Kerl, M.E., 2003. Update on canine and feline fungal diseases. Vet. Clin. North Am. Small Anim. Pract., 33: 721-747.

Khanum, S., Noor-us-Saba, M. Qayyum, Badar ul Islam and A.A. Qazilbash, 2006. Emergence of multi-drug resistant strains of *Salmonellatyphi* and *Paratyphi* A in the Rawalpindi/Islamabad. J. Medical Sci., 6: 68-73.

Kirsch, J.E., R.T. Greene, A. Prahl, S.I. Rubin, J.E. Sykes, M.M. Durkin and L.J. Wheat, 2012. Evaluation of coccidioides antigen detection in dogs with coccidioidomycosis. Clin. Vaccine. Immunol., 19: 343-345.

Kishi, K., T. Fujii, H. Takaya, A. Miyamoto, A. Kurosaki, T. Kohno and K. Yoshimura, 2008. Pulmonary coccidioidomycosis found in healthy Japanese individuals. Respirology, 13: 252-256.

Kumar, R., A.K. Verma, A. Kumar, M. Srivastava and H.P. Lal, 2012. Prevalence and Antibiogram of Campylobacter Infections in Dogs of Mathura, India. Asian J. Ani. Vet. Adv., 7: 434-440.

Lambourne, J., D. Agranoff, R. Herbrecht, P.F. Troke and A. Buchbinder *et al*., 2009. Association of mannose-binding lectin deficiency with acute invasive aspergillosis in immunocompromised patients. Clinic Infec. Dis., 49: 1486-1491.

Laniado-Laborin, R., J.M. Alcantar-Schramm and R. Cazares-Adame, 2012. Coccidioidomycosis: An update. Current Fung. Infec. Reports, 6: 113-120.

Luga, I.I., I. Akodu, L.I. Mhomga, L. Allam, I. Ajogi, V.J. Umoh and J.K.P. Kwaga, 2007. Antimicrobial resistance of shigatoxin producing *Escherichia coli* 0157:NM isolates from water fed to cattle in Northwestern Nigeria. Asian J. Anim. Vet. Adv., 2: 205-211.

Lupi, O., S.K. Tyring and M.R. McGinnis, 2005. Tropical dermatology: Fungal tropical diseases. J. Am. Acad. Dermatol., 53: 931-951.

Ma, Y.L., W.B. Zhang, B. Yu, Y.W. Chen, S. Mu and Y.L. Cui, 2011. Prevalence of allergic bronchopulmonary aspergillosis in Chinese patients with bronchial asthma. Zhonghua Jie He He Hu Xi Za Zhi, 34: 909-913 [In Chines].

Malabadi, R.B. and S.V. Kumar, 2007. Assessment of antifungal activity of some medicinal plants. Int. J. Pharmacol., 3: 499-504.

Malik, R., E. Dill-Macky, P. Martin, D.I. Wigney, D.B. Muir and D.N. Love, 1995. Cryptococcosis in dogs: A retrospective study of 20 consecutive cases. J. Med. Vet. Mycol., 33: 291-297.

McKinnell, J.A. and P.G. Pappas, 2009. Blastomycosis: New insights into diagnosis, prevention, and treatment. Clin. Chest Med., 30: 227-239.

McMillan, C.J. and S.M. Taylor, 2008. Transtracheal aspiration in the diagnosis of pulmonary blastomycosis (17 cases: 2000-2005). Can. Vet. J., 49: 53-55.

Mennink-Kersten, M.A., J.P. Donnelly and P.E. Verweij, 2004. Detection of circulating galactomannan for the diagnosis and management of invasive aspergillosis. Lancet Infect. Dis., 4: 349-357.

Moghim, S.H., M. Chalabi, A. Moghareh Abed, F. Rezaei and H. Tamizifar, 2007. Prevalence of epstein-barr virus type 1 in patients with chronic periodontitis by nested-PCR. Pak. J. Biol. Sci., 10: 4547-4550.

Momtaz, S. and M. Abdollahi, 2010. An update on pharmacology of *Satureja* species: From antioxidant, antimicrobial, antidiabetes and anti-hyperlipidemic to reproductive stimulation. Int. J. Pharmacol., 6: 346-353.

Oshin, A., D. Griffon, K. Lemberger, J. Naughton and A. Barger, 2009. Patellar blastomycosis in a dog. J. Am. Anim. Hosp. Assoc., 45: 239-244.

Oyella, J., D. Meya, F. Bajunirwe and M.R. Kamya, 2012. Prevalence and factors associated with cryptococcal antigenemia among severely immunosuppressed HIV-infected adults in Uganda: A cross-sectional study. J. Int. AIDS Soc. Vol. 15. 10.1186/1758-2652-15-15

Park, B.J., K. Sigel, V. Vaz, K. Komatsu and C McRill *et al*., 2005. An epidemic of coccidioidomycosis in Arizona associated with climatic changes, 1998-2001. J. Infect. Dis., 191: 1981-1987.

Patel, H., Y. Vaghasiya, B.R.M. Vyas and S. Chanda, 2012. Antibiotic-resistant *Staphylococcus aureus*: A challenge to researchers and clinicians. Bacteriol. J., 2: 23-45.

Peeters, D. and C. Clercx, 2007. Update on canine sinonasal aspergillosis. Vet. Clin. North Am. Small Anim. Pract., 37: 901-916.

Peeters, D., I.R. Peters, C.R. Helps, S. Dehard, M.J. Day and C. Clercx, 2008. Whole blood and tissue fungal DNA quantification in the diagnosis of canine sino-nasal aspergillosis. Vet. Microbiol., 128: 194-203.

Pfaller, M.A., G.J. Moet, S.A. Messer, R.N. Jones and M. Castanheira, 2011. Candida bloodstream infections: Comparison of species distributions and antifungal resistance patterns in community-onset and nosocomial isolates in the SENTRY antimicrobial surveillance program, 2008-2009. Antimicrob. Agents Chemother., 55: 561-566.

Pomrantz, J.S., L.R. Johnson, R.W. Nelson and E.R. Wisner, 2007. Comparison of serologic evaluation via agar gel immunodiffusion and fungal culture of tissue for diagnosis of nasal aspergillosis in dogs. J. Am. Vet. Med. Assoc., 230: 1319-1323.

Sarwar, M., I.H. Attitalla and M. Abdollahi, 2011. A review on the recent advances in pharmacological studies on medicinal plants: Animal studies are done but clinical studies needs completing. Asian J. Anim. Vet. Adv., 6: 867-883.

Sarwar, M., I.H. Attitalla and M. Abdollahi, 2011. A review on the recent advances in pharmacological studies on medicinal plants: Animal studies are done but clinical studies needs completing. Asian J. Anim. Vet. Adv., 6: 867-883.

Satoh, K., M. Maeda, Y. Umeda, Y. Miyajima and K. Makimura, 2011. Detection and identification of probable endemic fungal pathogen, *Cryptococcus gattii* and worldwide pathogen, *Cryptococcus neoformans*, by real-time PCR. Microbiol. Immunol., 55: 454-457.

Sidamonidze, K., M.K. Peck, M. Perez, D. Baumgardner, G. Smith, V. Chaturvedi and S. Chaturvedi, 2012. Real-time PCR assay for identification of *Blastomyces dermatitidis* in culture and in tissue. J. Clin. Microbiol., 50: 1783-1786.

Sidrim, J.J., A.K. Costa, R.A. Cordeiro, R.S. Brilhante and F.E. Moura *et al*., 2010. Molecular methods for the diagnosis and characterization of Cryptococcus: A review. Can. J. Microbiol., 56: 445-458.

Smith, J.A. and C.A. Kauffman, 2010. Blastomycosis. Proc. Am. Thorac. Soc., 7: 173-180.

Sohail, F. and M.N. Sohail, 2011. L. Despite of Its weedy nature is an important medicinal plant of NWFP, Pakistan. Int. J. Pharmacol., 7: 747-748.

Sohail, M.N., F. Rasul, A. Karim, U. Kanwal and I.H. Attitalla, 2011. Plant as a source of natural antiviral agents. Asian J. Anim. Vet. Adv., 6: 1125-1152.

Sohail, M.N., S.M. Karimi, S. Asad, S. Mansoor, Y. Zafar and Z. Mukhtar, 2012. Development of broad-spectrum insect-resistant tobacco by expression of synthetic cry1Ac and cry2Ab genes. Biotechnol. Lett., 34: 1553-1560.

Springer, D.J. and V. Chaturvedi, 2010. Projecting global occurrence of Cryptococcus gattii. Emerg. Infect. Dis., 16: 14-20.

Sumathi, C., S. Jayashree and G. Sekaran, 2012. Screening and evaluation of antimicrobial activity of tannery fleshing utilizing fish gut bacteria. Int. J. Pharmacol., 8: 373-381.

Thakur, R., S. Sarma and S. Kushwaha, 2008. Prevalence of HIV-associated cryptococcal meningitis and utility of microbiological determinants for its diagnosis in a tertiary care center. Indian J. Pathol. Microbiol., 51: 212-214.

Veron, V., S. Simon, D. Blanchet and C. Aznar, 2009. Real-time polymerase chain reaction detection of *Cryptococcus neoformans* and *Cryptococcus gattii* in human samples. Diagn. Microbiol. Infect. Dis., 65: 69-72.

Vucicevic, D., J.E. Blair, M.J. Binnicker, A.E. McCullough and S. Kusne *et al*., 2010. The utility of Coccidioides polymerase chain reaction testing in the clinical setting. Mycopathologia, 170: 345-351.

White, P.L., C. Mengoli, S. Bretagne, M. Cuenca-Estrella and N. Finnstrom *et al*., 2011. Evaluation of Aspergillus PCR protocols for testing serum specimens. J. Clin. Microbiol., 49: 3842-3848.

White, P.L., M.D. Perry, A. Moody, S.A. Follett, G. Morgan and R.A. Barnes, 2011. Evaluation of analytical and preliminary clinical performance of Myconostica MycAssay Aspergillus when testing serum specimens for diagnosis of invasive Aspergillosis. J. Clin. Microbiol., 49: 2169-2174.

Yousefi-Mashouf, R. and S.H. Hashemi, 2006. The epidemiology of burn wound infections in patients hospitalized in burn center of Hamedan, Western Iran. J. Med. Sci., 6: 426-431.

Zongo, C., A. Savadogo, L. Ouattara, I.H.N. Bassole and C.A.T. Ouattara *et al*., 2010. Polyphenols content, antioxidant and antimicrobial activities of *Ampelocissus grantii* (Baker) Planch. (Vitaceae): A medicinal plant from Burkina Faso. Int. J. Pharmacol., 6: 880-887.