Mini Review

Bordetella Bronchiseptica Infection and Kennel Cough in Dogs

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ABSTRACT

Among various pet animals, dogs have been men’s best companion and sharing the common dwellings. Recent analysis indicated that in India’s dog population has increased by 58% since 2007. Therefore, concern for diseases of dogs has also increased. The dogs often suffer from many diseases due to various managerial practices. One of such diseases is Bordetellosis often synonymous with kennel cough. Kennel cough is multi–etiologic disease but Bordetella bronchiseptica, a gram negative bacterium, has been considered the main causative agent. It causes canine infectious bronchitis characterized by frequent dry and hacking coughing with high morbidity (~ 80%). This organism has also reported to cause zoonotic infections in human beings. Though a well studied disease of dogs, little is understood about its epidemiology in developing countries including India. The PCR and ELISA are the common diagnostic methods for Bordetella bronchiseptica infection. However, detection of the pathogen does not mean the disease and many of the cases of kennel cough may not be associated with bordetellosis. Further studies are necessary to understand its epidemiology in developing countries for proper management of kennel cough and other related problems.

Key Words: Kennel–cough, Bordetellosis, B. bronchiseptica, Dog, India


Dog, an affectionate and loyal pet learns rapidly to live with its master with all his good and bad vices and is prone to acquire even the human diseases. Dogs’ population in India was around 10.2 millions in 2012 (Bradley and Kingno, 2012) and is increasing rapidly with change in socio–economic structure in India. In India, pup population increased by 58% during 2007 to 2012 (Euromonitor International). Increase in population, Dirofilaria UGH, is one of the etiologic disease but Kennel cough is multi–etiologic disease but Bordetella bronchiseptica, the causal organism of bordetellosis in dogs, is considered to be the main etiologic agent.

Kennel Cough

Canine infectious tracheobronchitis or kennel cough affects dogs of all ages. It is more common in dogs housed together in re–homing centres, boarding or training kennels, pet shops, shelters and veterinary clinics than in individually owned and stray dogs. The primary etiologies are Bordetella bronchiseptica, Canine Adenovirus (Bulut et al., 2013) and Canine Parainfluenza virus (Erles et al. 2004). Some secondary agents including Mammalian Reo virus, Canine respiratory Corona virus, CAV type 1 (CAV–1), Canine Herpes virus, Mycoplasma sp., Pseudomonas sp., Pasteurella sp., Streptococcus sp., and coliforms invading after some primary sickness may also induce the symptoms of kennel cough. More specifically, Streptococcus equi subspp. zoonedimicus and Mycoplasma cynos are often involved in causation of the disease which last much longer and more serious than the one caused by primary pathogens (Chalker et al., 2003 & 2004; Bucroft et al., 2007).

Bordetella bronchiseptica, a Gram–negative bacterium, colonizes the respiratory tract of wide range of mammalian hosts including dogs, pigs, cats, rabbits, mice, rats, guinea pigs, sheep, horses and bears (Lennox and Kelleher, 2009). The interspecies transmission of B. bronchiseptica has been reported among laboratory animals and between a rabbit and a human patient. It may be transmitted between cats and dogs living in close proximity and results in respiratory disease. It has already showed by few researchers that isolates from dogs and cats living in close proximity gave similar banding patterns in pulsed field gel electrophoresis.

Besides B. bronchiseptica, Canine Adenovirus type 2 is one of the other major etiologies of kennel cough (Bulut et al., 2013). The virus replicates in non–ciliated bronchiolar epithelial cells, nasal mucosa, pharynx, tonsillar crypts, mucous cells in the bronchi and trachea in peribronchial glands and type 2 alveolar epithelial cells which results in interstitial pneumonia, necrotizing bronchitis or bronchiolitis and bronchiolitis obliterans. For diagnosis of CAV 2 infection either cultivation

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of the virus in primary dog kidney cells or immunofluorescence assay (Buonavoglia and Martella, 2007) or polymerase chain reaction (Hu et al., 2001) or virus precipitation or hemagglutination inhibition or complement fixation or agar gel diffusion or virus neutralization tests have been used.

Another minor cause of kennel cough is Canine parainfluenza virus (Erles et al., 2004). For the detection of virus, hemadsorption or immunofluorescence, RT–PCR (Erles et al., 2004), hemagglutination inhibition and the virus neutralization tests have been demonstrated.

Rarely Canine Herpes virus (Kawakami et al., 2010), mammalian Rzo virus, Canine distemper virus and canine respiratory corona virus, S equi subsp. zoopneumoniae and Mycoplasma cynos (Chalker et al., 2003) may also be cause of kennel cough in dogs.

**Clinical Features of Bordetellosis**

Bordetellosis comprises of two clinical forms. The most common uncomplicated form is associated with dry hacking cough, gagging and retching behavior in dogs. The other form, the complicated form characterized by wet cough, is common in puppies or immuno-compromised dogs. The disease is associated with mucoid discharges and signs of systemic infection including pyrexia, anaemia, chorioretinitis, vomiting and diarrhoea leading to death of the pup. Incubation period ranges between 1 to 8 days showing clinical signs for 1–2 weeks. Infected dogs may shed the pathogen for 2–3 months after clinical recovery (Edinboro et al., 2004).

The genus *Bordetella*, belonging to family Alcaligenaceae (Gerlach et al., 2001), is comprised of nine species as *B. bronchiseptica*, *B. pertussis*, *B. parapertussis* (human), *B. parapertussis* (ovine), *B. hinzii*, *B. avium*, *B. holmsei*, *B. trematum* and *B. petrii* (Mattoo and Cherry, 2005). *Bordetella bronchiseptica* *B. pertussis*, *B. parapertussis* and *B. holmsei* and have been associated with zoonotic respiratory infections (Cotter and Miller, 2001).

*Bordetella bronchiseptica* had been known by many names earlier viz., *Bacillus bronchianis*, *Alcaligenes bronchisepticus*, *Brucella bronchiseptica*, *Alcaligenes bronchicanis*, *Haemophilus bronchisepticus* and finally Moreno–Lopez named it as *B. bronchiseptica*.

Besides kennel cough in dogs, *B. bronchiseptica* also causes atrophic rhinitis in pigs (Shome et al., 2008), snuffles in rabbits, suppurative bronchopneumonia in cats, suppurative necrotizing bronchopneumonia in guinea pigs, atrophic rhinitis in rats and respiratory infections in humans. Though *B. bronchiseptica* is a recognized cause of kennel cough throughout the world (Durgit et al., 2003), in India it is only a suspected cause of kennel cough and has rarely been isolated from dogs (Bonde et al., 1990; Reddy et al., 2003; Bhardwaj, 2013; Bhardwaj et al., 2013).

**Virulence Factors of Bordetella**

*Bordetella* LPS is a highly immunogenic, major constituent of the outer cell membrane and an important bacterial defense against host immune responses including antibodies, complement, antimicrobial peptides, and surfactants. The LPS of *B. bronchiseptica* is highly charged due to the presence of uronic acids in the O’ specific side chains, thus it is capable of masking negative charges present on the membranes to prevent an efficient membrane attack by the cationic antibacterial peptides (Preston and Maskell, 2001; Pilione et al., 2004; Schaeffer et al., 2004; Goebel et al., 2008).

The genus *Bordetella* exhibits several virulence factors such as adhesins, filamentous hemagglutinin, pertactin and fimbriae as well as the cytotoxic factor adenylyl cyclase toxin (ACT), which differ among different species. The expression of these virulence factors is controlled by the BvgAS two-component system in response to certain environmental stimuli. The regulatory system is characterized by antigenic modulation and phase variation. The antigenic modulation decides the activation and repression of synthesis of virulence factors which are dependent on growth conditions whereas phase variation is the result of mutations in *vir* gene which also modulates virulence under appropriate cultural conditions.

Filamentous hemagglutinin, a 220 kDa rod shaped protein encoded by *fhaB* gene, has been found to be associated with virulence (Mattoo and Cherry, 2005). However, adhesion proteins (Edwards et al., 2005) and pertactin (Sebaihia et al., 2006) are considered as important virulence factors for *Bordetella* species. Out of six major fimbrial subunits *fim2* and *fim3* fimbrial subunit genes are responsible for bordetellae adhesion to host cells (Mattoo and Cherry, 2005). The tracheal cytotoxin TCT expressed by *B. avium*, a toxin lethal for tracheal cells and degenerating bones is also present in *B. bronchiseptica*. The adenylyl cyclase toxin (ACT) also acts as an important factor for virulence of *Bordetella* strains (Masin et al., 2006; Vojnova et al., 2006; Buboltz et al., 2008). Besides all of these toxins, dermonecrotic toxin (DNT), a heat labile intracellular 160 kDa protein of *B. bronchiseptica* may play a role in the production of respiratory disease in dogs (Hoffmann and Schmidt, 2004; Masin et al., 2006).

**Diagnostic Techniques**

The diagnosis of bordetellosis depends primarily on the isolation of the *B. bronchiseptica* followed by the identification of the organism by biochemical, serological and molecular methods.

**Bacterial Culture and Isolation**

*Bordetella* species grow readily on blood agar, Bordet–Gengou agar, Smith–Baskerville culture media and MacConkey agar at optimum temperature of 37°C. The room temperature incubation should be avoided because of overgrowth of other bacteria suppressing *B. bronchiseptica* multiplication. In case of *B. pertussis* it was showed that colony numbers decreased by 75% on transportation of specimens at refrigerated temperature (4°C).

*Bordetella bronchiseptica* strains have phase 1 (smooth, small, convex and virulent) and phase 4 (rugged, large, and non-virulent) colonies. Biochemically, all strains are positive for oxidase, catalase and citrate utilization (Denes et al., 2006) and are negative for fermentation of any sugar, production of gelatinase, DNase, indole and H2S.

Isolation of *B. bronchiseptica* from different sites of respiratory tract of dogs (Gonzalez et al., 2006) and have widely been reported. But, in India the pathogen is rarely isolated from dogs (Bhardwaj, 2013; Bhardwaj et al., 2013). However, in India it has been isolated several times from pigs either associated with atrophic rhinitis or from healthy stocks (Shome et al., 2006; Mazumder et al., 2012; Kumar, 2013). But attempts have been made to isolate the organism from dogs (Bonde et al., 1990; Reddy et al., 2003). Bhardwaj (2013) reported isolation of *B. bronchiseptica* from an apparently healthy dog but not from the dogs suffering from kennel cough. They used transport media (buffered peptone water with 0.8% agar and horse serum) for transportation of nasal swabs and throat swabs. The isolate of *B. bronchiseptica* was sensitive to etrapenem, azithromycin, imipenem, ciprofloxacin, gentamicin, piperacillin-tazobactum, tetracycline polymixin-B and nalidixic whereas resistant to vancomycin, lincomycin, penicillin, cefotaxime, amoxicillin, ceftazidime, nitrofurantoin, ceftriaxone and amoxicillin+ clavulanic acid (Bhardwaj et al., 2013).
**Serological Methods**

Due to difficulty in isolation, serological tests are often considered good adjunct to facilitate diagnosis of kennel cough. Several serological tests have been developed, standardized and evaluated for detection of *Bordetella* antibodies for rapid assessment of prevalence of the infection in laboratory animals and other livestock. Commonly employed serological tests include tube agglutination, indirect haemagglutination (Bonde et al., 1990), micro-agglutination test (Denes, 2005; Kumar, 2013) and ELISA (Ellis et al., 2001; Kumar, 2013). In a study on 136 serum samples revealed that MAT titres positively correlate with kennel cough symptoms and were high in sick dogs than in apparently healthy dogs (Bhardwaj, 2013; Bhardwaj et al., 2013). High *Bordetella* agglutinin titre (128) in clinically diseased dogs indicates that *Bordetella* infection might be an important pathogen for kennel cough. Bhardwaj et al. (2013) also used whole cell ELISA (wcELISA) and precipitated protein ELISA (ppELISA) and found wcELISA much superior than ppELISA as an aid for diagnosis of bordetellosis in dogs.

**Molecular Diagnosis**

Isolation and identification of *B. bronchiseptica* is a time consuming process and serological techniques do not have good specificity. As a result, polymerase chain reaction (PCR) has been exploited to attain a fast and accurate detection of *Bordetella* in clinical samples. For the identification of *B. bronchiseptica*, genus specific and species specific PCRs (Hozbor et al., 1999; Coutinho et al., 2009; Register and Dejong, 2006; Koidl et al., 2007; Register and Nicholson, 2007; Xin et al., 2008; Steppenwolska and Markowska-Daniel, 2010; Roorda et al., 2012; Kumar, 2013; Bhardwaj et al., 2013) have been used. Ribotyping and RAPD analysis are already combined by a few researchers to evaluate genetic relatedness among canine *B. bronchiseptica* isolates.

Bhardwaj et al. (2013) screened 147 dogs using genus and species specific multiplex PCR but could not establish any good association between clinical disease and detection of *B. bronchiseptica* by PCR. The genus specific primers used are A643Bbac–F and A856Bbac–R (Table 1). Species specific primers used by different researchers (Table 2) have been designed either from *fim* or *flu* gene sequences.

**Table 1: List of genus–specific primers for Bordetella spp.**

<table>
<thead>
<tr>
<th>Name of primers</th>
<th>Sequence 5’ – 3’</th>
<th>Product length (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>A643Bbac–F</td>
<td>GCCGAGCCACCGCAGGAATAT</td>
<td>213</td>
<td>Bhardwaj, 2013; Kumar, 2013; Bhardwaj et al., 2013</td>
</tr>
<tr>
<td>A856Bbac–R</td>
<td>GGCGGTGACGAGATAGCTGTG</td>
<td>324</td>
<td></td>
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</tbody>
</table>

**Table 2: List of species–specific primers for Bordetella bronchiseptica**

<table>
<thead>
<tr>
<th>Name of primers</th>
<th>Sequence 5’ – 3’</th>
<th>Product length (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>425BBflm–1 F</td>
<td>TGAACCAATGGCAGTGAAGGC</td>
<td>425</td>
<td>Xin et al., 2008</td>
</tr>
<tr>
<td>425BBflm–2 R</td>
<td>TCGATAGTGGACGGGAGAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>237BBfla 4 F</td>
<td>TGGCGCTGGCCCTATC</td>
<td>237</td>
<td>Hozbor et al., 1999</td>
</tr>
<tr>
<td>237BBfla 2 R</td>
<td>AGGCCGCCAAGAGAAGGCTT</td>
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There are only few reports on plasmid profiling of *B. bronchiseptica* (Mazumder et al., 2012; Kumar, 2013). Bhardwaj et al. (2013) could identify single plasmid (Molecular weight, 50 MDa) from a single isolate of *B. bronchiseptica* while several isolates from pigs have been reported to harbor multiple plasmids (Mazumder et al., 2012; Kumar, 2013). Vaccines

Vaccination plays an important role in the prevention of infectious canine tracheobronchitis (Datz, 2003a). Live avirulent intranasal vaccines that combine *B. bronchiseptica* with canine parainfluenza virus, and canine adenovirus– 2, have been reported to confer better protection than *B. bronchiseptica* vaccines alone against kennel cough. Datz (2003b) reviewed modified live vaccines available worldwide for kennel cough intended for intranasal administration containing live, avirulent *B. bronchiseptica* with or without canine parainfluenza virus and canine adenovirus type 2. Intranasal as well as injectable vaccines of *B. bronchiseptica* may afford substantial protection against *B. bronchiseptica* (Ellis et al., 2003). In India, Reddy et al. (2003) prepared inactivated aluminium hydroxide gel *B. bronchiseptica* vaccine which protected vaccinated mice against homologous *B. bronchiseptica* challenge.

**Zoonotic Importance**

*Bordetella bronchiseptica* infection in humans is rare but has been documented in both healthy and immuno-suppressed individuals (Hewlett, 2000; Schneider and Gross, 2001; Lo et al., 2001). Intranasal vaccination in dogs may be one of the major risk factor for humans. Pneumonia, sepsis, and death have been reported after infection in human beings (Shimoni et al., 2000).

**CONCLUSIONS**

Kennel cough is one of the severe respiratory tract infections of dogs mostly in close confinement. Being a multi–etiologial disease, the identification of organism is a bit difficult. In developing countries including India, this disease has not been given much importance due to other important health problems in dogs and thus majority of bordetellosis cases might remain undiagnosed. This disease has major importance for dog breeders and army stations where they keep dogs in close association. This disease is of special concern due to its contagiousness. Due to difficulty in diagnosis, some rapid, reliable and economical tests need to be developed and evaluated to reveal its epidemiology in developing countries. Though PCR has been found to be the most rapid, sensitive (up to 5 CFU) and specific method to detect *B. bronchiseptica*, its field version needs evaluation. The MAT a well–evaluated test is better diagnostic test than ELISA but lack the desired sensitivity and specificity. Moreover, attempts for development of risk free vaccines are also needed to combat bordetellosis in dogs.
REFERENCES


