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Molecular identification and occurrence of bordetellosis among dogs in northern Kerala#

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Abstract

Bordetella bronchiseptica is a gram–negative bacterium with a broad host range that is most commonly seen in animals kept in close quarters. It is considered to be one of the main causes of the canine infectious respiratory disease complex (CIRDC). In this study, 45 dogs with clinical signs suggestive of CIRDC were selected and nasal/ oropharyngeal swabs were collected aseptically. Total DNA was extracted from the swabs and subjected to polymerase chain reaction (PCR) targeting alc gene of Bordetella spp. for confirmation. Nine animals (20 per cent) tested positive for the presence of Bordetella antigen. All the dogs tested positive for Bordetellosis were less than one year of age. Most common clinical signs in positive dogs were cough and nasal discharge, and the majority of the bordetellosis positive dogs were from multiple dog houses.

Keywords: *Bordetella bronchiseptica,* PCR, Occurrence, Kerala.

Dogs are frequently afflicted with a variety of diseases as a result of poor management practices. One major group of such diseases is the canine infectious respiratory disease complex (CIRDC), a group of contagious illnesses caused by bacteria and viruses. Affected animals will acquire moderate self-limiting clinical signs but some individuals, particularly immune compromised animals, will acquire significant clinical illness. These diseases are most typically encountered in boarding facilities, kennels, training centers, shelters and other places where dogs are confined together (Mitchell *et al.*, 2017).

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Primary etiologies of CIRDC are *Bordetella bronchiseptica*, canine adenovirus and canine parainfluenza virus, mammalian reovirus, canine respiratory coronavirus, canine herpes virus, *Mycoplasma* spp., *Pseudomonas* spp., *Pasteurella* spp., *Streptococcus* spp. with coliforms being considered as secondary pathogens (Reagan and Skyes, 2020).

The primary causal agent of CIRDC is believed to be *B. bronchiseptica,* a gram–negative bacterium. Gram-negative bacterial disease is a major public health concern around the world (Swetha *et al.,* 2022). Besides, this organism has been associated with human zoonotic infections. Despite the fact that this is a well-studied disease among dogs, sparse information is available on the epidemiology in our country.

Materials and methods

A total of 45 dogs showing clinical signs suggestive of CIRDC formed the sample of the study. Information regarding age, breed, sex, management details and vaccination history was collected. Nasal swabs were collected from all dogs aseptically using sterile cotton swabs (Hi-Media, Mumbai). Swabs collected were cut and put in an Eppendorf tubes containing 300µl PBS (pH 7) and stored at -20°C.

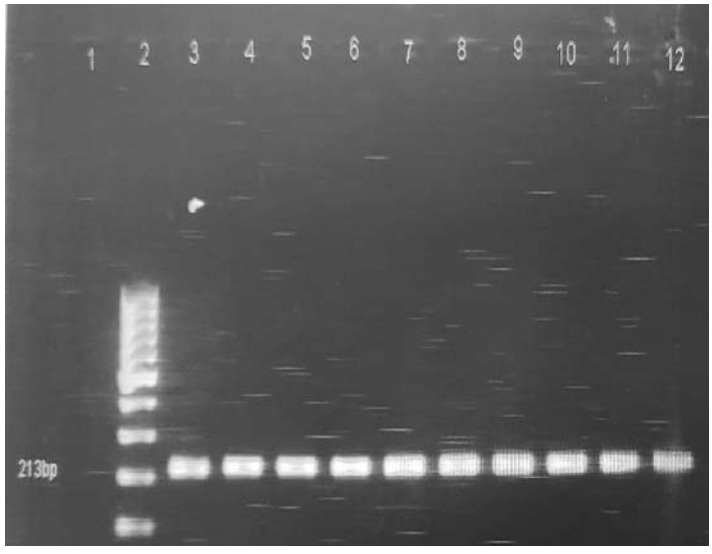
These samples were further subjected to DNA isolation with the DNeasy Blood and Tissue Kit (M/s Qiagen inc. Catalog no. 69504 and 69506). These isolated DNA were subjected to polymerase chain reaction targeting *alc* gene of *Bordetella* spp. (Bhardwaj *et al.*, 2013). Standardisation of PCR was done using DNA isolated from Nobivac ® KC live vaccine as positive control and nuclease free water as negative control. Thermal cycling conditions comprised of an initial denaturation at 95°C for 10 min, followed by 35 cycles of 94°C for 30 sec, 53°C for 30 sec and 72°C for 45 sec. The final extension was performed at 72°C for 7 min (Bhardwaj *et al.*, 2013).

PCR amplicons were sequenced to ensure their exact identification and thus the PCR reaction specificity. Representative positive PCR products were sequenced at AgriGenome Lab Private Limited, Cochin. The sequences were then analysed using FinchTV software and homology checked using NCBI-BLAST.

Results and discussion

Molecular detection by PCR targeting 213 bp sized amplicons of *alc* gene was considered positive for bordetellosis. Sequencing and BLAST analysis of

Fig. 1. Amplicons of *Bordetella bronchiseptica*



(Lane 1- negative control; Lane 2- 100bp DNA ladder; Lane 3- Positive control; Lane 4- 12- positive samples)

amplicons showed 100 per cent identity with *B. bronchiseptica.* In this study, 20 per cent of clinically ill dogs were conformed for bordetellosis by PCR (Fig .1). Similar results were obtained by (Steinfeld *et al.* 2012) who recorded a prevalence of 20.2 per cent for bordetellosis on PCR among the dogs of Germany.

All dogs positive for bordetellosis belonged to less than one year age group. This result is in agreement with the findings of Mochizuki *et al.* (2008), who reported that bordetellosis was found mainly among puppies aged two to three months. According to these authors, the puppies were primarily exposed to infections during group breeding and became ill soon after being introduced to their new owners.

Among the nine dogs positive for bordetellosis, cough and nasal discharge were the predominant clinical signs observed. These findings are consistent with the findings of Schulz *et al.*(2013), who found that cough (86.7 percent) was the most common clinical symptom in Bordetella positive dogs followed by nasal discharge (62.2 percent). This organism adheres to the cilia along and produce toxins leading to ciliostasis of respiratory epithelial cells and further aids in secondary infections by other pathogens. The organism can enter and survive within inflammatory cells and affect the immune system of the host (Edwards *et al.,* 2005).

Majority of the dog's positive for bordetellosis were from multiple dog houses. This result is in accordance with the finding of Bhardwaj *et al.* (2013) who stated that CIRDC was found to be more common in dogs confined together in kennels, shelters re–homing facilities, veterinary clinics and pet shops than in dogs that were singly owned or stray.

The present study indicated the presence of *B. bronchiseptica* among young dog population especially under poor nutrition and management condition. The mortality rate due to B. *bronchiseptica* is low and it is a self-limiting disease unless the animal is immunocompromised. The study assumes significance as little is known about its epidemiology in India.

Conclusion

Bordetella bronchiseptica is one of the major pathogens associated with upper respiratory disease among dogs and the results of present study highlights the need to include Bordetella vaccine in the canine vaccination regimen.

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Conflict of interest

The authors declare that there is no conflict of interest in publishing this paper.

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