ISOLATION OF BACTERIAL PATHOGENS ASSOCIATED WITH CANINE RESPIRATORY DISEASE

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ABSTRACT

The present study was conducted to identify the bacterial pathogens involved in respiratory diseases in dogs. Nasal samples from upper respiratory tract were collected using sterile swabs from the canine clinical cases that were presented with the history of respiratory diseases in Veterinary Clinical Complex, College of Veterinary Sciences, LUVAS over a period of 6 months from July (2018) to December (2018). Paired nasal swabs were taken from 36 dogs and immediately transferred aseptically into a nutrient broth and were subjected to bacteriological examination. Purified isolates were subjected to various cultural tests and microscopic examination. All the nasal swabs were found to be positive for bacterial organisms and different species of bacteria isolated includes *Streptococcus* spp. (30.7%), *Staphylococcus* spp. (28.8%), *Salmonella* spp. (11.5%), *E. coli* (11.5%), *Klebsiella* spp. (5.76%), *Bacillus* spp. (5.76%), *Pseudomonas* spp. (3.8%) and least found isolate was *Actinomyces* spp. (1.9%).

Keywords: Bacteriological examination, Canine respiratory disease, Microscopic examination, Nasal swabs

Respiratory diseases in dogs are associated with various etiological agents such as bacterial, viral, parasitic and others. Among these agents, respiratory disease due to bacterial etiology is common in dogs (Epstein et al., 2010). The clinical manifestations associated with canine respiratory diseases appear suddenly and sometimes they may be chronic, refractory and resistant to commonly employed antibiotics (Adaszek et al., 2009). There is a need to formulate procedures for the clinical management of respiratory diseases in dogs in order to achieve quick therapeutic response and also to alleviate pain and suffering. Respiratory disease is a challenging condition to diagnose (Centil et al., 2012) and to treat as patients can exhibit a wide range of clinical presentations ranging from dyspnoea, costal or abdominal respiration, cough, nasal discharge and congestion, edema, consolidation of lungs, lethargy, weight loss and ultimately fatal syndrome. Bacteria involved in respiratory infections in dogs and cats are usually Escherichia coli, Klebsiella spp., Streptococcus spp. and Staphylococcus spp. strains (Attili et al., 2012). Infectious diseases of the respiratory tract caused by Staphylococcus spp. result in severe conditions and sometimes become fatal in pups. Bordetella bronchiseptica (Sykes, 2009) and Pseudomonas spp. were also noticed in samples collected from respiratory infections (Angus et al., 1997). Some canine cases of bacterial pneumonia upon culture examination of transtracheal aspiration samples revealed the presence of Mycoplasma spp. and aerobic bacteria (Attili et al., 2012).

The objective of this study was to isolate the various bacterial etiological agents associated with respiratory

tract infection in dogs.

MATERIALS AND METHODS

Study area: All the samples was collected from clinical cases reported in small medicine section of VCC, LUVAS, Hisar. Sampling period was from July 2018 to December 2018.

Bacteriological examination: Nasal swabs were taken aseptically from the affected animals and streaked on 5% Sheep blood agar (BA) and MacConkey's lactose agar (MLA) plates, with the help of a 4 mm diameter platinum loop. The plates were incubated aerobically at 37 °C for 24 to 48 hours. Sub-cultures of the resulting growth were made on blood agar for purification of isolates and identified on the basis of Gram's reaction, morphology and colony characteristics. Some other media had also been used for identification of bacterial species-XLD (Xylose lysine Deoxycholate) to observe black colour colonies of Salmonella spp. and BGA (Brilliant Green Agar) culture media used for identification of pink colour colonies of Salmonella spp. EMB (Eosine Methylene Blue) used for identification of metallic sheen of E. coli. Nutrient agar used to identify medusa head colonies of *Bacillus* spp. and to observe green and blue colour pigmentation of *Pseudomonas* spp.

RESULTS AND DISCUSSION

During cultural examination of nasal swab of 36 dogs affected with respiratory diseases, all dogs were found to be positive with isolation of 52 different organisms. The different organism isolated were *Streptococcus* spp. (Fig. 1), *Staphylococcus* spp. (Fig. 2), *E. coli* (Fig. 3), *Salmonella* spp. (Fig. 4 and 5), *Bacillus*

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Table 1

Relative frequency of organism isolated from Nasal swab samples of dogs (n=36) suffering from respiratory disease

Organisms isolated	Frequency (%)
Single infection	21 (58.3)
Streptococcus spp.	16(30.7)
Staphylococcus spp.	15 (28.8)
Salmonella spp.	6(11.5)
E. coli	6(11.5)
Klebsiella spp.	3 (5.8)
Bacillus spp.	3 (5.8)
Pseudomonas spp.	2(3.8)
Actinomyces spp.	1(1.9)
Mixed Infection	15 (41.6)
Staphylococcus spp. + Streptococcus spp.	2 (3.8)
Staphylococcus spp. + E. coli	2(3.8)
Staphylococcus spp. + Salmonella spp.	1(1.9)
<i>Staphylococcus</i> spp. + <i>E</i> . <i>coli</i> + <i>Bacillus</i> spp.	1(1.9)
Streptococcus spp. + Klebsiella spp.	2(3.8)
$Streptococcus \operatorname{spp.} + E. coli$	2(3.8)
Streptococcus spp. + Salmonella spp.	4(7.6)
Streptococcus spp. + Pseudomonas spp.	1(1.9)
Total	52

spp. (Fig. 6), *Klebsiella* spp. (Fig. 7), *Pseudomonas* spp. (Fig. 8) and *Actinomyces* spp. (Fig. 9) either alone or in different combination as shown in Table 1. Mixed infections included combination of *Staphylococcus* spp. and *E. coli*, *Staphylococcus* spp. and *Streptococcus* spp., *Staphylococcus* spp., and *Salmonella* spp., *Staphylococcus* spp. and *Klebsiella* spp., *Streptococcus* spp. and *Klebsiella* spp., *Streptococcus* spp. and *Salmonella* spp., *Streptococcus* spp. and *Pseudomonas* spp.

This study investigated detection rates of bacterial isolates in 36 samples of dogs having respiratory tract infection. Bacterial species most frequently isolated were Streptococcus spp. (30.7%) and our findings are well supported by the results of Christina et al. (2010) who found heavy growth of beta-haemolytic Streptococci, Staphylococcus spp. and Klebsiella spp. from the bacterial cultures of the nasal swab from dogs. Similar prevalence was also reported by Rheinwald et al. (2015) by observing Streptococcus spp. to be the most frequent isolate from respiratory tract infected dogs. Our findings for Streptococcus spp. as the most common isolate are also well supported by Peeters et al. (2000) who also observed Streptococcus spp. to be the most common isolate during his study on cases of chronic bronchitis in dogs. In contrary to our study with respect to Streptococcus spp. as most common isolate, Johnson *et al.* (2013) observed *Mycoplasma* spp. as the most common isolate having no isolation of *Streptococcus* spp. during his study on respiratory tract infection in dogs.

In our study, *Staphylococcus* spp. has been isolated with a frequency of 28.8% and our findings are well supported by Morrissey *et al.* (2016) who conducted a prevalence study during time period between 2008 and 2010 on respiratory tract infections associated with bacterial isolates in dogs and observed *Staphylococcus intermedius* group (22.8%) as most common isolates.

As E. coli has been isolated in our study and similar findings are also observed in an older study (Angus et al., 1997) who observed that the most prevalent species were E. coli, Pasteurella spp. and Streptococcus spp. Our findings for isolates of E. coli are also well supported by Adaszek et al. (2009), which demonstrated that the isolated bacteria in canine respiratory infections were usually E. coli, Klebsiella spp. and Staphylococcus spp. Ayodhya et al. (2013) during their study on canine respiratory diseases also observed that the bacteria such as E. coli, Klebsiella spp., Streptococci spp., Staphylococcus spp., and mixed infections are the common causative agents for canine respiratory diseases. In our study, our frequency of E. coli isolates is 11.5% and similar findings for E. coli isolates having a frequency of 11.2% by Morrissey et al. (2016) and 11% by Epstein et al. (2010) has been observed on respiratory tract infections in dogs.

Our findings for isolates of *Klebsiella* spp. are well supported by Ayodhya *et al.* (2013), Adaszek *et al.* (2009), Proulx *et al.* (2014), Durgut *et al.* (2003). In our findings, *Klebsiella* spp. was observed with a frequency of 5.76% which is in accordance with the results observed by Epstein *et al.* (2010) with a frequency of 4% for *Klebsiella* spp. in a study of 84 dogs having respiratory disease.

In our study, *Pseudomonas* spp. has also been isolated from respiratory tract infected dogs and our results of *Pseudomonas* spp. isolates are supported by Johnson *et al.* (2013), Basso *et al.* (2009), Rheinwald *et al.* (2015) and Peeters *et al.* (2000). Proulx *et al.* (2014) also observed *Pseudomonas* spp. isolates with a low frequency (5.3%) which is close to our findings having 3.8% frequency of *Pseudomonas* spp. isolates. While in contrary to our study with respect to *Pseudomonas* spp. isolates. While in contrary to a study with respect to *Pseudomonas* spp. isolates are supplication, Ayodhya *et al.* (2013), Epstein *et al.* (2010), Morrissey *et al.* (2016) during bacterial isolation from respiratory tract infected dogs did not observe any isolates of *Pseudomonas* spp.

For *Bacillus* spp. and *Actinomyces* spp. isolates, our findings are similar to findings of Peeters *et al.* (2000). They conducted study to determine the usefulness of



Fig. 1. Dew drop hemolytic colonies of *Streptococcus* I spp. on blood agar

Fig. 2. Golden colour colonies of *Staphylococcus* spp. Fig. 3. on blood agar

Metallic sheen of *E. coli* on Eosine methylene blue (EMB) agar







Fig. 4. Pink colour colonies of *Salmonella* spp. on Fig. 5. Brilliant Green Agar

- . Black colour colonies of *Salmonella* spp. on Fig. 6. Xylose Lysine Deoxcholate (XLD) agar
- Medussa head colonies of *Bacillus* spp. on Nutrient agar



Fig. 7.Mucoid pink colonies of Klebsieela spp. on
Mac Conkey Lactose agarFig. 8.Green colour pigmentation of Pseudomonas
spp. on Nutrient agarFig. 9.White coloured non-hemolytic colonies of
Actinomyces spp. on Blood agar

quantitative aerobic cultures and Gram stain intracellular bacteria counts from bronchoalveolar lavage (BAL) specimens in dogs in diagnosing lower respiratory tract infection (LRTI) and to determine whether chronic bronchitis is associated with marked bacterial growth in dogs.

There are several explanations for the differences in the detection rates between these previous studies and this one. First, there might be differences in the dog populations studied. Secondly, differences in the microbiological spectrum of the isolates could also be influenced by the different timeframes and different geographical locations in which the studies were carried out.

CONCLUSION

Our study revealed isolation of bacteria from clinical cases of respiratory infection in dogs. *Streptococus* spp. was the most common isolated bacteria followed by *Staphylococcus* spp. Present study highlights the prevalence of bacteria in nasal passage of dogs which can cause diseases in human beings owing to close association of humans with their pets.

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