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RESEARCH ARTICLE

Genotypic Characterization of *Bordetella bronchiseptica* Strains Isolated from Stray and Pet Dogs

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ABSTRACT

Bordetella bronchiseptica (*B. bronchiseptica*) is the most important pathogen associated with kennel cough in dogs. The presence of *B. bronchiseptica* in pet dogs and shelter dogs with clinical respiratory disease was investigated in present study. The genetic relatedness among the strains was determined to evaluate the role of stray dogs in spread of *B. bronchiseptica* to pet dogs by detection of virulence genes such as filamentous hemagglutinin (*fha*), pertactin (*prn*) and dermonecrotic toxin (*dnt*). We also performed the random amplified polymorphic DNA (RAPD) assay. A total of 96 *B. bronchiseptica* were isolated from stray and pet dogs. The *fha*, *prn* and *dnt* virulence genes were detected in 86, 83.3 and 61.4% strains, respectively by polymerase chain reaction (PCR) techniques. The most common genotype from stray and pet dogs was *fha*+*prn*+*dnt*+ as detected in 37.5% and 11.4% of all the strains, respectively. The RAPD assay showed that 3 different patterns were obtained from 96 *B. bronchiseptica* strains. Sixty one (63.5%) of them were clustered in one main group and then further placed in another 2 sub-groups by RAPD assay. Genetic association was seen between the *B. bronchiseptica* strains from stray and pet dogs. In conclusion, this study revealed that *B. bronchiseptica* is present at a higher rate in stray dogs than pet dogs. Stray dogs might have a significant role in the transmission of *B. bronchiseptica* to pet dogs.

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INTRODUCTION

Bordetella bronchiseptica is a zoonotic and one of the major respiratory pathogen which causes infectious tracheobronchitis, commonly known as kennel cough in dogs (Bhardwaj *et al*., 2013; Kalhoro *et al*., 2015). The disease mostly occurs when a large numbers of dogs are housed together in pet shops, in breeding or boarding kennels, in shelters, in research facilities and even in veterinary clinics (Bhardwaj *et al*., 2013). Infection with *B. bronchiseptica* can increase the pathogenicity of different viral and bacterial pathogens by destroying the ciliated epithelial cells (Register, 2004). Attachment to ciliated respiratory epithelial cells is a key event in *Bordetella* pathogenesis (Zhaoa *et al*., 2009). *Bordetella* spp. produce a variety of adhesins such as *fha*, *prn* and fimbriae as well as toxins such as *dnt*, adenylatecyclase and tracheal cytotoxin. All of these are associated with bacterial attachment, colonization and tissue damage (Stepniewska and Daniel, 2010). The *fha* and *prn* are the major adhesin and colonization factor of the *Bordetella* species. They also induce a protective immune response and used to develop several vaccine formulations (Bock and Gross, 2001). The *dnt* of *B. bronchiseptica* which is also called heat-labile toxin causes skin lesions and has vasoconstrictive activity. It also induces mucosal damage in swine nasal tissue and causes turbinate atrophy in pigs. However, the role of *dnt* is not clear in canine respiratory infections (Bock and Gross, 2001; Brockmeier and Register, 2007). The PCR detection of virulence genes can be used as markers for pathogenicity and can provide a rapid genotypic characterization of *B. bronchiseptica* strains (Stepniewska and Daniel, 2010, 2012; Stepniewska *et al.*, 2014).

Overpopulation of stray dogs is a major problem in Turkey (Ucan and Aras, 2015). Via a national legislative process, stray dogs are captured, vaccinated, neutered and kept at most 3 months in shelters. They are released to the environment from which they were originally taken. However, no routine vaccination schedule against *B. bronchiseptica* in these shelters is applied. There are very limited data on presence of the *B. bronchiseptica* infection in dogs in Turkey (Maden *et al*., 2000; Erdeger, 2002). Here, our goal was to investigate the presence of *B. bronchiseptica* in pet dogs and shelter dogs with clinical respiratory disease in Turkey. In addition, genetic relatedness among *B. bronchiseptica* strains was identified to evaluate the role of stray dogs in the transmission of *B. bronchiseptica* to pet dogs by detection of virulence factor genes such as *fha*, *prn*, *dnt* and RAPD-PCR analysis.

MATERIALS AND METHODS

Sampling and Culture: The bronchoalveolar lavage fluid (BALF) samples were collected from 126 stray dogs kept in shelter houses from Konya Municipality, Turkey as well as 36 pet dogs from the Faculty of Veterinary Medicine, Selcuk University, Konya. All the animals included in this study showed at least one of the clinical signs of respiratory infection such as fever, respiratory rate, cough, nasal discharge, increased respiratory rate, and abnormal bronchial sounds. The BALF samples were taken by passing a nasotracheal tube and then infusing 10 mL of sterile saline solution under local anesthesia with lidocaine. We also collected 12 lung samples by necropsy of dogs with respiratory disease that died at the shelter. All samples were inoculated onto Bordet Gengou agar (Difco, USA) supplemented with 15% defibrinated horse blood and 10 ml/L (v/v) glycerol. These were incubated aerobically at 37°C for 48 h as previously described (Larson *et al*., 2013). Typical, flat, non-hemolytic, oxidase and catalase positive colonies were further identified by conventional biochemical tests such as motility, growth on Mac Conkey agar, urease, citrate utilization, nitrate reduction, oxidation/fermentation of glucose and xylose (Winn *et al*., 2006). The strains identified as *B. bronchiseptica* were stored in sterile skim milk at -80°C.

PCR analysis: Chromosomal DNA from *B. bronchiseptica* strains were extracted from overnight cultures in 2 mL Brain Heart Infusion Broth (Difco, USA) using Wizard Genomic DNA purification Kit (Promega, Cat. numb. A1120, USA) according to the manufacturer's instructions. The DNA samples were stored at -20°C until PCR analysis. Two primer sets targeting the upstream region of the *fla* gene were used for the identification of *B. bronchiseptica* (Table 1). One was a genus-specific primer set (Fla1/Fla2) for *Bordetella* spp., and the other was a species-specific primer set (Fla2/Fla4) for *B. bronchiseptica* (Hozbor *et al*., 1999). The PCR cycling parameters were as follows: initial denaturation at 95°C for 10 min, then 35 cycles at at 94°C for 15 sec, 53°C for 15 sec, 72°C for 20 sec, and 72°C for 10 min for each gene. The DNA from *B. bronchiseptica* ATCC 4617 and sterile distilled water were the positive and negative controls in PCR analysis, respectively. Three sets of primers were used for detection of *fha*, *prn*, (Zhaoa *et al*., 2009) and *dnt* genes (Stepniewska and Daniel, 2010) in *B. bronchiseptica* strains (Table 1).

RAPD-PCR analysis: The RAPD analysis of *B. bronchiseptica* strains was performed as described by Keil and Fenwick (1999) and Shin *et al*. (2007) and using OPA-4 primer (Table 1). The Total Lab Quant Analysis Software (UK) program was used to analyze the DNA bands and construct dendograms.

Statistical analysis: The statistical analyses were performed using SPSS software version 12. The *B. bronchiseptica* isolation rate comparisons between stray and pet dogs were performed using the *Chi-Square* ($\chi 2$) test at $P<0.05$.

RESULTS

Culture: A total 96 *B. bronchiseptica* were isolated from 162 BALF samples and 12 lung samples. Of these, 86 were isolated from BALF samples, 6 from 126 shelter dogs (52.3%) and 20 strains from 58 (34.4%) pet dogs. Ten pulmonary strains of the 12 (83.3%) obtained from stray dogs died of pneumonia. The isolation rate of *B. bronchiseptica* was higher in stray dogs (55%, n=76) than that of the pet dogs (34.4%, n=20). The difference was statistically significant ($P<0.05$).

PCR analysis: All 96 strains which were identified as *B. bronchiseptica* by biochemical test gave positive bands at the 164 bp of the *fla* gene in *Bordetella* spp. genus-specific PCR analysis. They produced 237 bp amplicons and were confirmed as *B. bronchiseptica* via species specific-PCR analysis.

Virulence genes: The *fha, prn*, and *dnt* virulence genes of *B. bronchiseptica* were detected in 83 (86%), 80 (83.3%), and 59 (61.4%) strains by PCR analysis, respectively. Based on presence or absence of tested virulence genes seven different genotypes were detected in *B. bronchiseptica* strains (Table 2). The most common genotype was the one included all the genes of *fha, prn* and *dnt* as evidenced by 47 (48.9%) of *B. bronchiseptica* strains in this genotype. Thirty (37.5%) of these were isolated from shelter dogs and 11 (11.4%) were from pet dogs. Distribution of this genotype based on the origins of dogs was 47.3% and 55% in shelter and pet dogs, respectively (Table 2). The unique presence of either genes *fha* or *dnt* were detected in 8 strains only. The genotypes with two genes were detected in 25 (*fha*+*prn*+*dnt*-), 3 (*fha*+*prn*-*dnt*+) and 8 (*fha*-*prn*+*dnt*+) *B. bronchiseptica* strains. Four number of strains originated from shelter dogs showed the genotype of *fha*-*prn*-*dnt*-. The specific genotype of the isolate carrying only *dnt* gene detected from just one shelter dog.

RAPD-PCR analysis: The genetic relationship among all RAPD patterns of *B. bronchiseptica* is represented in the UPGMA dendogram shown in Figure 1. Four distinct RAPD patterns were obtained from 96 *B. bronchiseptica* strains, and each type contained one to three PCR products ranging in size from 400 to 1800 bp with a fragment of 400 bp detected in all the strains. All *B.*

bronchiseptica strains were put into 3 separate clusters (I, II and III in Figure 1, Table 3) in a dendogram. A total of 63.5% (n=61) of the strains were clustered in one main group (I in Figure 1) and further placed in 2 sub-groups (A, B) of the UPGMA dendogram. Fifteen of these strains were placed in sub-group A and 46 in sub-group B. The other 35 strains were located in two main groups (II and III in Figure 1, Table 3). There is an obviously genetic association between shelter dogs and pet dogs in terms of *B. bronchiseptica* strains. A high percentage of the *B. bronchiseptica* strains based on the dogs' origin 46%, (n=35) for the shelter dogs and 55%, (n=11) for the pet dogs were found in the same cluster (sub-group B of cluster I). The 36 of 47 (76.5%) strains possesing *fha+prn+dnt+* genotype detected by PCR were also placed in sub-group B as measured by RAPD-PCR.

DISCUSSION

Uncontrolled and overpopulated packs of stray dogs can transmit many infections to humans and other animals including life-threatening diseases such as rabies as well as less threating diseases like *B. bronchiseptica* infections (Anonymous, 2015). The agent is a common inhabitant of the respiratory tract of several animal species, and one of the main microorganism of the kennel cough in dogs particularly in crowded shelters (Chalker *et al*., 2003; Bhardwaj *et al*., 2013). To date, there are few studies on the presence of *B. bronchiseptica*on in dogs and no data regarding characterization of strains in Turkey (Maden *et al*., 2000; Erdeger, 2002).

Table 1: PCR primers used in the study

Name of primers	Primer sequences 5'-3'	Amplicon size (bp)
Fla1	5'-CCCCCGCACATTTCCGAACTTC-3'	164
Fla2	5' AGGCTCCCAAGAGAGAAAGGCTT-3'	237
Fla4	5'-TGGCGCCTGCCCTATC-3'	
fha	F-5'-TTTAAGAATTCCTGACTGCCCTGGACAAT-3' R-5'-TTTAAGTCGACTCGCAGATCCGCGGCAAA-3'	465
prn	F-5'-TAATTGTCGACAACACCATGCTGCTGGTG-3' R-5'-TTTAACTGCAGGGCGGACAACTCCCTGCC-3'	765
dnt	F-5'-GCGGTACTTGGGATAATAGA-3' R-5'-ATAAAGATGAATCGGCATTG-3'	224
OPA-4	5'-AATCGGGCTG-3'	Variable

Table 2: Virulence gene profiles of *B. bronchiseptica* strains

Genotypes	Number of strains		
	Shelter dogs n (%)	Pet dogs n (%)	Total n (%)
fha+prn+dnt+	36 (37.5)	11 (11.4)	47 (48.9)
fha+prn+dnt-	20 (20.8)	5 (5.2)	25 (26)
fha+prn-dnt-	6 (6.2)	2 (2)	8 (8.3)
fha+prn-dnt+	3 (3.1)	-	3 (3.1)
fha-prn-dnt-	4 (4.1)	-	4 (4.1)
fha-prn+dnt+	6 (6.2)	2 (2)	8 (8.3)
fha-prn-dnt+	1 (1)	-	1 (1)
Total	76	20	96

(**+**): gene is present in strain, (**-**): gene is absent in strain

Table 3: RAPD patterns of *B. bronchiseptica* strains.

RAPD type No	Number of strains			PCR product size(s) (bp)
	Shelter dogs	Pet dogs	Total (%)	
I	44	17	61 (63.5)	400 to 1800
Sub-group A	11	4	15 (15.6)	400-1800
†Sub-group B	35	11	46 (47.9)	400-800-1800
II	17	3	20 (20.8)	400-800
III	14	1	15 (15.6)	400

†The most common identical RAPD pattern.

In this study, presence of *B. bronchiseptica* was investigated in shelter dogs and pet dogs from Konya that located in the center of Anatolia. Genetic relatedness among strains was detected using RAPD-PCR analysis. Some genes related to virulence factors such as *fha*, *prn*, and *dnt* were also detected by PCR in the strains to determine possible effects of stray dogs on the transmission of *B. bronchiseptica* to pets in this city.

A total 96 (48.7%) *B. bronchiseptica* were isolated from 196 samples and identified by standard microbiological methods and PCR. A higher isolation rate was observed for the shelter dogs (76 of 138; 55%) by comparison with pet dogs (20 of 58; 34.4%), ($P < 0.05$). These findings are consistent with the results of previous studies from Turkey and the World (Erdeğer, 2002; Chalker *et al*., 2003; Shulz *et al.,* 2014). Erdeger (2002) isolated *B. bronchiseptica* from 8 of 13 (61%) diseased and 10 of 119 (8.4%) shelter dogs that were healthy by clinical examination in Turkey. In another study, a low isolation rate (24%) in canine BALF samples was seen (Maden *et al.*, 2000). However, the characteristics of the sampled dogs (pet or shelter dogs) are not clearly stated in that study. This suggests that comparing our study and this prior study is difficult. A high occurrence of *B. bronchiseptica* isolation in shelter dogs in our study can be explained by the fact that lower isolated and overcrowded areas serves a good medium for spreading contagious respiratory infections. The lower presence of the infection in pet dogs sampled by this study may be because of status of protection as triggered by vaccination.

The expression of Bordetella virulence determinants is controlled by Bordetella virulence gene (*bvg*) locus (Stepniewska and Daniel, 2012). The presence of Bordetella virulence genes has been used as a marker for pathogenicity as reported many times (Brockmeier *et al*., 2002; Zhaoa *et al.*, 2009; Inatsuka *et al.*, 2010). It readily determines the genotypic profiles of strains (Register, 2004; Friedman *et al.*, 2006; Stepniewska and Daniel, 2012; Khayer *et al.*, 2014; Stepniewska *et al.*, 2014). In the present study, seven different *B. bronchiseptica* genotypes were determined based on the occurrence of virulence genes, and a similar result was reported by a recent study reporting that *B. bronchiseptica* strains isolated from swine (Stepniewska and Daniel, 2012). They used flagella (*fla*) and exogenous ferric siderophore receptor (*bfrZ*) genes in addition to the *dnt* gene. Most *B. bronchiseptica* strains from shelter (47.3%) or households (55%) were detected within the same genotype (*fha+prn+dnt+*). We believe that these results suggest the role of shelter dogs on transmission of *B. bronchiseptica* to pet dogs. This is further supported by a prior study that determined *B. bronchiseptica* in a dead pet dog's body after a period of keeping in a private shelter (Bagcıgil *et al*., 2007).

It is known by the previous studies that virulence genes such as *fha*, *prn* and *dnt* are necessary for tracheal colonization and pathogenicity of Bordetella (Brockmier *et al*., 2002; Zhaoa *et al*., 2009) although some conflicting results from others have suggested that these are not obligatory for the maintenance of its pathogenicity because mutant strains are not completely avirulent (Godwin and Weiss, 1990; Brockmier and Register, 2007). In a previous study, Brockmeier *et al*. (2002)

showed that *dnt* knockout mutants of *B. bronchiseptica* did not cause pneumonia or atrophic rhinitis in pigs. Later, they observed a similar magnitude of turbinate atrophy in pigs initially inoculated with either the wild type or the *dnt* mutant of *B. bronchiseptica* and challenged with *P. multocida* (Brockmeier *et al*., 2007). In some mouse models, no differences were observed between *fha* mutants and wild type *B. bronchiseptica* strains for tracheal colonization capacity (Goodwin and Weiss, 1990).

The strain ratio of 48.9% as found by this study showed presence of the genes of all three. We did not observed any virulence genes from very small number of *B. bronchiseptica* strains. In agreement with previous studies (Godwin and Weiss, 1990; Brockmeier and Register, 2007), these results showed that virulence genes also tested by this study are not considered as essential genetic elements for pathogenicity of *B. bronchiseptica* strains.

The detection of DNA polymorphism by RAPD fingerprinting was used successfully for many bacteria including *Bordetella* spp. to provide information about epidemiological relationships between strains (Keil and Fenwick, 1999; Shin *et al*., 2007). In our study, 4 distinct RAPD-PCR patterns from 96 *B. bronchiseptica* strains, and three different sized PCR products (400, 800 and 18000 bp) were detected by the OP-4 primer. These findings are consistent with the results of Keil and Fenwick (1999) who used the same primer pair for typing *B. bronchiseptica* strains isolated from canines. However, they obtained different PCR products (861 bp to 2.8 kb). The polymorphism among *B. bronchiseptica* strains may be because of deletion or insertion mutations. On the other hand, Shin *et al*. (2007) reported 7 distinct RAPD fingerprint patterns in 41 swine *B. bronchiseptica* strains using the same primer (OPA-4). These authors suggested that differences might be due to the existence of a host species-specific *B. bronchiseptica* because an earlier study produced a diverse finding (Keil and Fenwick, 1999).

Although 3 different RAPD-genotypes may be present in this study, only one predominant type (cluster I) causes disease in shelter and pet dogs. We found that 46% and 55% of the *B. bronchiseptica* strains isolated from shelter dogs and pet dogs are located in the same cluster (sub-group B of cluster I), respectively. Also, most of the strains (76.5%) showed same genotypic characteristic according to both presence of virulence genes and RAPD-PCR profiles. Our results indicate that there is a strong genetic relationship between shelter and pet dogs in terms of *B. bronchiseptica* strains. These findings are consistent with the results of other researchers (Keil and Fenwick, 1999; Shin *et al*., 2007) who have demonstrated that the population structure of *Bordetella* spp. is clonal with little genetic diversity.

In conclusion, this study demonstrated that *B. bronchiseptica* is present at a higher rate in the shelter dogs than pet dogs with respiratory disease in Konya, Turkey. Stray dogs might have a significant role in the transmission of *B. bronchiseptica* to pet dogs if they share a common environment.

This study previously presented partly as an oral presentation at the XI. Veterinary Microbiology Congress (October 2014-Antalya/Turkey). This research has been approved (2014/59) by the Ethics Committee of the Faculty of Veterinary Medicine at the University of Selcuk in Konya, Turkey.

Author's contribution: AC collected the BALF samples from dogs. ZS, AS, OE, USU, HHH, ZA, GS carried out microbiological and PCR analyses of *B.bronchiseptica* isolates. All authors interpreted the data, critically revised the manuscript and approved the final version.

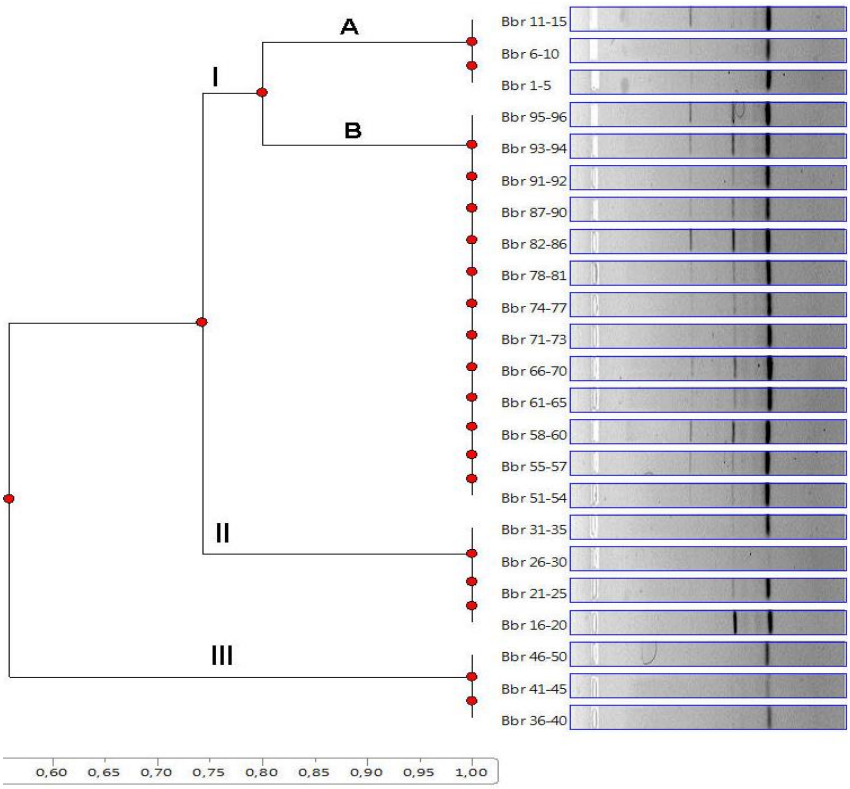

Fig. 1: Genetic relationship among the 96 *B. bronchiseptica* strains from two types of origins was estimated by clustering analysis of RAPD patterns. The dendogram was generated by the UPGMA method.

REFERENCES

Anonymous, 2015. Stray dog population control. In: Terrestrial Animal Health Code. Chap. 7. 7, pp. 1-2. World Organisation for Animal Health, Paris, France.

Bagcıgil AF, Sennazli G, Metiner K and Yıldız F, 2007. A case of death caused by *Bordetalla bronchiseptica* in a dog. J Fac Vet Med Istanbul Univ, 33: 75-83.

Bhardwaj M, Singh BR and Vadhana P, 2013. *Bordetella bronchiseptica* infection and Kennel Cough in dogs. Adv Anim Vet Sci, 1: 1-4.

Bock A and Gross R, 2001. The BvgAS two-component system of *Bordetella* spp.: a versatile modulator of virulence gene expression. Int J Med Microbiol, 291: 119-130.

Brockmeier SL, Register KB, Magyar T, Lax AJ, Pullinger GD, *et al*., 2002. Role of the dermonecrotictoxin of *Bordetella bronchiseptica* in the pathogenesis of respiratory disease in swine. Infect Immun, 70: 481-490.

Brockmeier SL and Register KB, 2007. Expression of the dermonecrotic toxin by *Bordetella bronchiseptica* is not necessary for predisposing to infection with toxigenic *Pasteurella multocida*. Vet Microbiol, 125: 284-289.

Chalker VJ, Toomey C, Opperman S, Brooks HW, Ibuoye MA, *et al*., 2003. Respiratory disease in kennelled dogs: Serological responses to *Bordetella bronchiseptica* lipopolysaccharide do not correlate with bacterial isolation or clinical respiratory symptoms. Clin Diagn Lab Immunol, 10: 352-356.

Erdeger J, 2002. Isolation of *Bordetella bronchiseptica* from upper respiratory tracts of stray dogs. Ankara Univ Vet Fak Derg, 49: 119-123.

Friedman LE, Messina MT, Santoferrara L, Santillan MA, Mangano A, *et al*., 2006. Characterization of *Bordetella bronchiseptica* strains using phenotypic and genotypic markers. Vet Microbiol, 117: 313-320.

Goodwin MS and Weiss AA, 1990. Adenylate cyclase toxin is critical for colonization and pertussis toxin is critical for lethal infection by *Bordetella pertussis* in infant mice. Infect Immun, 58: 3445-3447.

Hozbor D, Fouque F and Guiso N, 1999. Detection of *Bordetella bronchiseptica* by the polymerase chain reaction. Res Microbiol, 150: 333-341.

Inatsuka CS, Xu Q, Vujkovic-Cvijin I, Wong S, Stibitz S, *et al*., 2010. Pertactin is required for Bordetella species to resist neutrophil-mediated clearance. Infect Immun, 7: 2901-2909.

Kalhoro DH, Luo S, Xie X, Zhao YB, Lu CP, *et al*., 2015. *Streptococcus pluranimalium* isolated from a canine respiratory case: identification and experimental infection in mice. Pak Vet J, 35: 388-390.

Keil DJ and Fenwick B, 1999. Evaluation of canine *Bordetella bronchiseptica* isolates using randomly amplified polymorphic DNA fingerprinting and ribotyping. Vet Microbiol, 66: 41-51.

Khayer B, Magyar T and Wehmann E, 2014. Flagellin typing of *Bordetella bronchiseptica* strains originating from different host species. Vet Microbiol, 173: 270-278.

Larson LJ, Thiel BE, Sharp P and Schultz RD, 2013. A comparative study of protective immunity provided by oral, intranasal and parenteral canine *Bordetella bronchiseptica* vaccines. Int J Appl Res Vet Med, 11: 153-160.

Maden M, Birdane FM, Alkan F, Hadimli HH, Sen I, *et al*., 2000. Clinical, cytologic, bacteriologic and radiographic analysis of respiratory diseases in dogs. Eurasian J Vet Sci, 16: 43-50.

Register KB, 2004. Comparative sequence analysis of *Bordetella bronchiseptica* pertactin gene (*prn*) repeat region variants in swine vaccines and field isolates. Vaccine, 23: 48-57.

Shin EK, Seo YS, Han JH and Hahn TW, 2007. Diversity of swine *Bordetella bronchiseptica* isolates evaluated by RAPD analysis and PFGE. J Vet Sci, 8: 65-73.

Schulz BS, Kurz S, Weber K, Balzer HJ and Hartmann K, 2014. Detection of respiratory viruses and *Bordetella bronchiseptica* in dogs with acute respiratory tract infections. Vet J, 201: 365-369.

Stepniewska K and Daniel MI, 2010. Evaluation of PCR test for detection of dermonecrotoxin of *Bordetella bronchiseptica*. Bull Vet Inst Pulawy, 54: 495-499.

Stepniewska K and Daniel MI, 2012. Occurence of genes encoding virulence factors in *Bordetella bronchiseptica* strains isolated from infected and healthy pigs. Bull Vet Inst Pulawy, 56: 483-487.

Stepniewska K, Urbaniak K and Daniel MI, 2014. Phenotypic and genotypic characterization of *Bordetella bronchiseptica* strains isolated from pigs in Poland. Pol J Vet Sci, 17: 71-77.

Ucan US and Aras Z, 2015. The "*One Health*" concept and a need for national monitoring of canine and feline infections. Proc "1st Turkish Congress of One Health", Konya, Turkey; 9-10 April, 2015, pp: 61-62.

Winn W, Allen S, Janda W, Koneman, E, Procop G, *et al*., 2006. Bordetella species. In: Koneman's Color Atlas and Textbook of Diagnostic Microbiology (Darcy P, ed). 6th Ed, Williams&Wilkins, Philadelphia, USA, pp: 510-522.

Zhaoa Z, Xuea Y, Tanga X, Wua B, Cheng X, *et al*., 2009. Immunogenicity of recombinant protective antigen and efficacy against intranasal challenge with *Bordetella bronchiseptica.* Vaccine, 27: 2523-2528.