Molecular detection of canine respiratory pathogens between 2017 and 2018 in Japan

Aya Matsuu¹*, Mihoko Yabuki¹, Emiko Aoki², Michio Iwahana²

¹Transboundary Animal Diseases Research Center, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan

²Zoetis Japan Co., Ltd., 3-22-7 Yoyogi, Shibuya, Tokyo 151-0053, Japan

*Corresponding author: Aya Matsuu

Transboundary Animal Diseases Research Center, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan

Tel and Fax: +81-99-285-3611

E-mail: matsuu@vet.kagoshima-u.ac.jp

Running head: CIRD BETWEEN 2017 AND 2018 IN JAPAN
ABSTRACT

A molecular survey was conducted to understand recent distribution of pathogens associated with canine infectious respiratory disease (CIRD) in Japan. Nasal and/or pharyngeal swabs were collected from asymptomatic dogs and those with CIRD, living in private house or in kennels. PCR-based examination was conducted for detecting nine pathogens. Among private household dogs, 50.8% with CIRD, 11.1% with respiratory disease other than CIRD, and 4.3% asymptomatic were positive for more than one pathogen, whereas in kennel-housed dogs, 42.9% with CIRD and 27.3% asymptomatic were positive. *Bordetella bronchiseptica* was most frequently detected, followed by canine herpesvirus 1, canine parainfluenza virus, canine pneumovirus, *Mycoplasma cynos*, and canine adenovirus type 2. In kennel environment, asymptomatic dogs might act as reservoirs carrying the respiratory pathogens.

KEYWORDS: asymptomatic, canine infectious respiratory disease, dog, kennel environment
Canine infectious respiratory disease (CIRD), classically known as “kennel cough”, is a common syndrome of dogs, especially young ones that are kept in large groups, as in shelters, pet shop, or breeding kennels. CIRD may occur even in pet dogs housed at home. Typical clinical signs include acute onset of coughing and nasal discharge, sometimes dyspnea with or without fever. CIRD is considered a complex infection with multifactorial etiology, associated with classical and newly emerging pathogens [10, 24].

Canine distemper virus (CDV), canine parainfluenza virus (CPiV), canine adenovirus type-2 (CAV-2), and *Bordetella bronchiseptica* are well known classical pathogens of CIRD [24] that are primarily associated with respiratory diseases in dogs. In addition, canine herpesvirus type 1 (CHV-1) [16], canine pneumovirus (CPnV) [20, 25, 26], canine respiratory coronavirus (CRCoV) [5, 21, 27, 34], *Mycoplasma cynos* [13], canine influenza virus (CIV) [11, 22], *Streptococcus equi* subsp. *zooepidemicus* [17, 23], canine bocavirus [15, 24], and canine hepacivirus [1, 7] are some recently identified emerging agents. In recent molecular epidemiological studies, the prevalence of these new and emerging respiratory pathogens in CIRD dogs have been reported. In Italy, it was reported that CPiV was mainly responsible for CIRD occurrences, followed by CRCoV, *B. bronchiseptica*, *M. cynos*, *M. canis*, and CPnV, whereas classical CIRD
agents, such as CAV and CDV were not detected at all. In another study from New Zealand, CIRD dogs were found mostly positive for *M. cynos*, followed by CPiV, and *B. bronchiseptica*, and all dogs were found negative for CIV, CRCoV, CDV, and *S. equi* subsp. *zooepidemics*. Although CIRD is the most common, worldwide, its etiology or epidemiology may vary across countries [6, 12, 18, 19, 21, 28, 31].

In 2008, an etiologic study of upper respiratory infections of house dogs in Japan had reported *B. bronchiseptica* and CPiV as the principal etiologic agents [21]. Another study in 2009 reported *B. bronchiseptica*, CPiV, and CRCoV to possibly be the major pathogens in CIRD [29]. Prevalence of some pathogens, including CIV, CPnV, and *M. cynos* has not been elucidated till date. A more recent epidemiologic survey and the relative prevalence in Japanese dog population would be useful for veterinary practice and animal health sciences. Here, we conducted a molecular etiology-based study using samples from symptomatic and asymptomatic dogs living in two different environments.

Between 2017 and 2018, nasal and/or pharyngeal swab samples were collected from 167 household dogs, and submitted by 52 animal hospitals from 27 prefectures throughout Japan. Based on the information provided by the veterinarians, 61 dogs were suspected to have CIRD owing to acute onset (< 1 week) of cough with or without
fever, 83 dogs showed respiratory signs, although not CIRD (chronic cough since over a month, heart failure, or tracheal collapse), and 23 dogs were asymptomatic. Some dogs with respiratory symptoms had been already administered antibiotic products at the time of sampling. Other swab samples were collected from 80 dogs housed in 12 kennels from 12 prefectures. Among these dogs, 14 showed clinical signs of CIRD, 66 were asymptomatic, and no dogs showed chronic respiratory signs. Detailed animal information is summarized in Table 1. Swabs were placed immediately in BD universal viral transport medium (Becton, Dickinson and Company, NJ, USA). Total nucleotide was extracted from the supernatant using an automatic extraction system magLEAD® with magLEAD® Dx SV reagent (Precision System Science Co., Ltd., Tokyo, Japan), according to the manufacturer’s instructions. This study was approved by the Institutional Animal Care and Use committee, Kagoshima University (permission number: VM19045), and was carried out according to the guidelines of the committee.

For detection of CHV and *B. bronchiseptica* DNA, TaqMan-probe real-time PCR (THUNDERBIRD probe qPCR Mix, TOYOBO, Osaka, Japan) was conducted using the CHV-specific primer sets (Forward: 5′-ACAGAGTTGATTGATAGAAGGTATG-3′, Reverse: 5′-CTGGTGTATTAAACTTTGAAGGCTTTA-3′) and a probe (FAM-5′-
bronchiseptica-specific primer sets (Forward: 5’-AGGCTCCCAAGAGAGAAGGCTT-3’, Reverse: 5’-AAACCTGCCGAATCCAGGC-3’) and a probe (5’-FAM-ACCGGGCAGCTAGGCCTG-TAMRA-3’) [32], respectively, according to previous studies. For CPnV detection, TaqMan-probe real-time RT-PCR (THUNDERBIRD probe one-step qRT-PCR Mix, TOYOBO) was conducted using the CPnV-specific primer sets (Forward: 5’- GACCTGTTTGAAAGGAAGCCTTATT-3’, Reverse: 5’- ACCAGAAAACAGCCCCTCAAC-3’) and a probe (5’-FAM-CTTCCATCATTTTGGCCTGGCCCAG-TAMRA-3’) [20]. For detection of CPiV, CDV, and CIV RNA, SYBR green real-time RT-PCR (Brilliant III Ultra-Fast SYBR Green QPCR Master Mixes, Agilent Technologies, CA, USA) was performed using specific primer sets for CaPiV (Forward: ATATGGCGGCGTGATTAAAG, and Reverse: TGAATCATTCGATTGCCAAA) [21], CDV (Forward: 5’-AGCTAGTTTCATCTTTAACTATCAAATT-3’, Reverse: 5’-TTAACTCTCCAGAAAACCTCATGC-3’) [8], and CIV (Forward: 5’-CCMAGGTCGAAACGTAYGTTCTCTCTATC-3’, Reverse: 5’-TGACAGRATYGGTCTTGTCTTAGCCAYTCCA-3’) as in previous studies. For
CRCoV detection, gel-based nested RT-PCR, using the first primer set (Forward: 5’-TATCGCAGCCTTTACTTTTGT-3’, and Reverse: 5’-ACCGCCGTATGGTTATCAG-3’), followed by a second PCR, using another primer set (Forward: 5’-GCACAATCTACAGCTCTTTG-3’, and Reverse: 5’-AGACAGATTGCTTTCGTAGGA-3’) [34], was performed. For the detection of *M. cynos* DNA, gel-based conventional PCR was performed, using a specific primer set (Forward: 5’-CACCGCCCGTCACACCA-3’, and Reverse: 5’-GATACATAAACACAACATTATAATATTG-3’) as in a previous study [13].

In private household dogs, 70.5% with CIRD were young, under 6 months of age. The proportion of age was compared by χ² testing, and significant differences were seen among dogs with CIRD, without CIRD, and those asymptomatic (*P* < 0.01). There was no significant difference in the proportions of gender, environment, presence of innate animals, and vaccination history.

In private household dogs, 31 out of 61 (50.8%) with CIRD, 9 out of 83 (10.8%) with respiratory diseases other than CIRD, and 1 out of 23 (4.3%) asymptomatic dogs were positive for more than one pathogen. On the other hand, in kennel-housed dogs, 4 out of 14 (28.6%) with CIRD and 18 out of 66 (27.3%) asymptomatic ones were positive for more than one pathogen. The number of positive
mono- or co-infections is shown in Table 2, and the number of positive individual pathogens is shown in Table 3. Among the private household dogs, *B. bronchiseptica* was the most frequently detected (40.3%), followed by CHV1 (7.5%), CPIV (4.5%), *M. cynos* (4.5%), CaPnV (4.5%), and CAV-2 (1.5%) amongst the ones with CIRD. In dogs with respiratory diseases other than CIRD, *B. bronchiseptica* (9.6%) and CHV (1.2%) were detected. *B. bronchiseptica* was detected in an asymptomatic dog as well (4.3%). In dogs with CIRD, co-infection of more than two pathogens was detected in 11 cases (18.0%). Among kennel-housed dogs, *B. bronchiseptica* (28.6%), and *M. cynos* (14.3%) were detected in the ones with CIRD, whereas *B. bronchiseptica* (16.7%), CHV-1 (3.0%), CPnV (6.1%), and *M. cynos* (1.5%) were detected in asymptomatic ones. Co-infection of *B. bronchiseptica* and *M. cynos* was detected in 2 dogs (14.3%) with CIRD. In this study, CDV, CrCoV, and CIV were not detected in any grouped dog.

CIRD is a common disease of dogs worldwide. In the past decade, several studies have reported the prevalence of newly emerging as well as traditional CIRD pathogens [6, 18, 19], and the results vary widely across countries [24]. In general, *B. bronchiseptica*, CPIV, CDV, and CAV-2 have been considered the main agents of CIRD [2, 9, 21]. In our current study, CPIV and CAV-2 nucleic acids were detected in 4.5% and 1.5% of private household dogs with CIRD, whereas CDV nucleic acid was not.
a previous study in Japan, in 2008 [21], CPiV, CAV-2, and CDV nucleic acids were detected in 7.4%, 2.9%, and 1.5% of household dogs, respectively. The extensive vaccination programs might have maintained a low circulation of these viruses in the dog population in Japan. However, *B. bronchiseptica* was the most frequently detected agent in private household dogs with CIRD. This pathogen was detected not only in dogs with CIRD but also in those with respiratory diseases other than CIRD. This suggested that *B. bronchiseptica* circulates widely across Japan, and causes primary CIRD as well as secondary infection leading to other respiratory diseases. Dogs in kennel environment could be sub-clinical carriers, acting as a source of infection for susceptible dogs. *B. bronchiseptica* can act as the primary CIRD pathogen, and its pathogenic potential is increased with simultaneous viral and bacterial infections [24]. Ten out of 61 dogs with CIRD, among private house dogs, showed co-infection of *B. bronchiseptica* with other pathogens, and this frequency was significantly higher than in other groups by χ² testing (P < 0.01). Although inactivated intranasal *B. bronchiseptica* vaccine, combined with CPiV and CAV-2, is commercially available in Japan, no vaccinated dog in this study population had been administered with this product. To control this pathogen more efficiently, more positive administration would be recommended.
In our knowledge, prevalence of *M. cynos* and CPnV among the dog population in Japan has not been described till date. In our present study, 4.5% private household dogs with CIRD were found positive for *M. cynos*, with the same prevalence of CPnV. These pathogens were detected from asymptomatic kennel-housed dogs, thereby indicating that they mainly circulate among kennel or sheltered environment just like *B. bronchiseptica*. Because some of the symptomatic private household dogs had been already administered antibiotics at the sampling time, the actual positive rate of *B. bronchiseptica* and *M. cynos* might be higher than this result.

CRCoV and CIV nucleic acids were not detected in this study. CRCoV infection had been confirmed in previous studies with Japanese household dogs, and the detection rate in dogs with CIRD was 1.5% [21] and 16.0% [29], respectively. Recent studies from European countries have also reported high prevalence of CCoRV [19, 28]. Reasons for the negative results of CRCoV in our study remain unclear; continuous study using bigger population might be necessary. Till date, several subtypes of type A influenza virus have been isolated from dogs, which have caused CIRD in them across USA, China, Korea, and European countries [3, 14, 18, 30, 33]. These viruses have not been detected in Japan till date; therefore, CIV seems to not be an important pathogen so far for dogs in Japan.
In this study, we collected swab samples from dogs, throughout Japan. We found that there was no obvious geographical distribution of pathogens. Additionally, we collected kennel samples from 80 dogs of 12 kennels, where *B. bronchiseptica* was detected from 15 dogs in 4 kennels, CaPnV was detected from 4 dogs in one kennel, and *M. cynos* was detected from 3 dogs in another kennel (data not shown). These pathogens seem to spread in a facility with a dense population.

In conclusion, we have demonstrated *B. bronchiseptica* to be the most frequently detected pathogen among dogs with CIRD in Japan, followed by CHV-1, CPIV, CPnV, *M. cynos*, and CAV-2. Considering the increasing association of emerging viral and bacterial pathogens, continuous and frequent surveillance studies, using a larger population, is needed, which might eventually be useful for specific vaccination and treatment programs.

**Acknowledgements**

We would like to acknowledge the assistance of all the participating veterinarians and their staff.

**Conflict of Interest statement**

This study was funded by Zoetis Japan Co., Ltd. Aoki and Iwahana are employees of
REFERENCES

1. Bukh, J. 2011. Hepatitis C homolog in dogs with respiratory illness. *Proc. Natl. Acad. Sci. USA* **108**: 12563–12564.

2. Buonavoglia, C. and Martella, V. 2007. Canine respiratory viruses. *Vet. Res.* **38**: 355–373.

3. Crawford, P. C., Dubovi, E. J., Castleman, W. L., Stephenson, I., Gibbs, E. P., Chen, L., Smith, C., Hill, R. C., Ferro, P., Pompey, J., Bright, R. A., Medina, M. J., Johnson, C. M., Olsen, C. W., Cox, N. J., Klimov, A. I., Katz, J. M. and Donis, R. O. 2005. Transmission of equine influenza virus to dogs. *Science* **310**: 482–485.

4. Decaro, N., Amorisco, F., Desario, C., Lorusso, E., Camero, M., Bellacicco, A. L., Sciarretta, R., Lucente, M. S., Martella, V. and Buonavoglia, C. 2010. Development and validation of a real-time PCR assay for specific and sensitive detection of canid herpesvirus 1. *J. Virol.* **Methods** **169**: 176–180.

5. Decaro, N., Desario, C., Elia, G., Mari, V., Lucente, M. S., Cordioli, P., Colaianni, M. L., Martella, V. and Buonavoglia, C. 2007. Serological and molecular evidence that canine respiratory coronavirus is circulating in Italy. *Vet. Microbiol.* **121**: 225–230.

6. Decaro, N., Mari, V., Larocca, V., Losurdo, M., Lanave, G., Lucente, M. S., Corrente, M., Catella, C., Bo, S., Elia, G., Torre, G., Grandolfo, E., Martella, V. and Buonavoglia, C. 2016. Molecular surveillance of traditional and emerging pathogens associated with canine
infectious respiratory disease. *Vet. Microbiol.* **192**: 21–25.

7. El-Attar, L. M., Mitchell, J. A., Brooks Brownlie, H., Priestnall, S. L. and Brownlie, J. 2015. Detection of non-primate hepaciviruses in UK dogs. *Virology* **484**: 93–102.

8. Elia, G., Decaro, N., Martella, V., Cirone, F., Lucente, M. S., Lorusso, E., Di Trani, L. and Buonavoglia, C. 2006. Detection of canine distemper virus in dogs by real-time RT-PCR. *J. Virol. Methods* **136**: 171–176.

9. Erles, K., Dubovi, E. J., Brooks, H. W. and Brownlie, J. 2004. Longitudinal study of viruses associated with canine infectious respiratory disease. *J. Clin. Microbiol.* **42**: 4524–4529.

10. Ford, R. B. 2012. Canine infectious respiratory disease. pp. 55–67. In: Greene, C. E. (Ed.)’s Infectious Diseases of the Dog and Cat, 4th ed. Elsevier (Saunders), St Louis, MO, USA.

11. Harder, T. C. and Vahlenkamp, T. W. 2010. Influenza virus infections in dogs and cats. *Vet. Immunol. Immunopathol.* **134**: 54–60.

12. Hiebl, A., Auer, A., Bagrinovschi, G., Stejskal, M., Hirt, R., Rümenapf, H. T., Tichy, A. and Künzel, F. 2019. Detection of selected viral pathogens in dogs with canine infectious respiratory disease in Austria. *J. Small Anim. Pract.* **60**: 594–600.

13. Hong, S. and Kim, O. 2012. Molecular identification of *Mycoplasma cynos* from
laboratory beagle dogs with respiratory disease. *Lab. Anim. Res.* **28**: 61–66.

14. Jeoung, H. Y., Lim, S. I., Shin, B. H., Lim, J. A., Song, J. Y., Song, D. S., Kang, B. K., Moon, H. J. and An, D. J. 2013. A novel canine influenza H3N2 virus isolated from cats in an animal shelter. *Vet. Microbiol.* **165**: 281–286.

15. Kapoor, A., Mehta, N., Dubovi, E. J., Simmonds, P., Govindasamy, L., Medina, J. L., Street, C., Shields, S. and Lipkin, W. I. 2012. Characterization of novel canine bocaviruses and their association with respiratory disease. *J. Gen. Virol.* **93**: 341–346.

16. Kawakami, K., Ogawa, H., Maeda, K., Imai, A., Ohashi, E., Matsunaga, S., Tohya, Y., Ohshima, T. and Mochizuki, M. 2010. Nosocomial outbreak of serious canine infectious tracheobronchitis (kennel cough) caused by canine herpesvirus infection. *J. Clin. Microbiol.* **48**: 1176–1181.

17. Ladlow, J., Scase, T. and Waller, A. 2006. Canine strangles case reveals a new host susceptible to infection with *Streptococcus equi*. *J. Clin. Microbiol.* **44**: 2664–2665.

18. Lavan, R. and Knesl, O. 2015. Prevalence of canine infectious respiratory pathogens in asymptomatic dogs presented at US animal shelters. *J. Small Anim. Pract.* **56**: 572–576.

19. Mitchell, J. A., Cardwell, J. M., Leach, H., Walker, C. A., Le Poder, S., Decaro, N., Rusvai, M., Egberink, H., Rottier, P., Fernandez, M., Fragkiadaki, E., Shields, S. and Brownlie, J. 2017. European surveillance of emerging pathogens associated with canine infectious
respiratory disease. *Vet. Microbiol.* **212**: 31–38.

20. Mitchell, J. A., Cardwell, J. M., Renshaw, R. W., Dubovi, E. J. and Brownlie, J. 2013. Detection of canine pneumovirus in dogs with canine infectious respiratory disease. *J. Clin. Microbiol.* **51**: 4112–4119.

21. Mochizuki, M., Yachi, A., Ohshima, T., Ohuchi, A. and Ishida, T. 2008. Etiologic study of upper respiratory infections of household dogs. *J. Vet. Med. Sci.* **70**: 563–569.

22. Parrish, C. R. and Voorhees, I. E. H. 2019. H3N8 and H3N2 canine influenza viruses: Understanding these new viruses in dogs. *Vet. Clin. North Am. Small Anim. Pract.* **49**: 643–649.

23. Pesavento, P. A., Hurley, K. F., Bannasch, M. J., Artiushin, S. and Timoney, J. F. 2008. A clonal outbreak of acute fatal hemorrhagic pneumonia in intensively housed (shelter) dogs caused by *Streptococcus equi* subsp. zooepidemicus. *Vet. Pathol.* **45**: 51–53.

24. Priestnall, S. L., Mitchell, J. A., Walker, C. A., Erles, K. and Brownlie, J. 2014. New and emerging pathogens in canine infectious respiratory disease. *Vet. Pathol.* **51**: 492–504.

25. Renshaw, R., Laverack, M., Zylitch, N., Glaser, A. and Dubovi, E. 2011. Genomic analysis of a pneumovirus isolated from dogs with acute respiratory disease. *Vet. Microbiol.* **150**: 88–95.

26. Renshaw, R. W., Zylitch, N. C., Laverack, M. A., Glaser, A. L. and Dubovi, E. J. 2010.
Pneumovirus in dogs with acute respiratory disease. Emerg. Infect. Dis. 16: 993–995.

27. Soma, T., Ishii, H., Miyata, K. and Hara, M. 2008. Prevalence of antibodies to canine respiratory coronavirus in some dog populations in Japan. Vet. Rec. 163: 368–369.

28. Schulz, B. S., Kurz, S., Weber, K., Balzer, H. J. and Hartmann, K. 2014. Detection of respiratory viruses and Bordetella bronchiseptica in dogs with acute respiratory tract infections. Vet. J. 201: 365–369.

29. Sehata, G., Wakatsuki, A., Masubuchi, K., Takahashi, T. and Kokubu, T. 2010. Etiological investigation of canine infectious respiratory disease in Japan. J. Jpn. Vet. Med. Assoc. 63: 538–542.

30. Song, D., Moon, H. J., An, D. J., Jeoung, H. Y., Kim, H., Yeom, M. J., Hong, M., Nam, J., H., Park, S. J., Park, B. K., Oh, J. S., Song, M., Webster, R. G., Kim, J. K. and Kang, B. K. 2012. A novel reassortant canine H3N1 influenza virus between pandemic H1N1 and canine H3N2 influenza viruses in Korea. J. Gen. Virol. 93: 551–554.

31. Sowman, H. R., Cave, N. J. and Dunowska, M. 2018. A survey of canine respiratory pathogens in New Zealand dogs. N. Z. Vet. J. 66: 236–242.

32. Tizolova, A., Brun, D., Guiso, N. and Guillot, S. 2014. Development of real-time PCR assay for differential detection of Bordetella bronchiseptica and Bordetella parapertussis. Diagn. Microbiol. Infect. Dis. 78: 347–351.
33. Voorhees, I. E. H., Glaser, A. L., Toohey-Kurth, K., Newbury, S., Dalziel, B. D., Dubovi, E. J., Poulsen, K., Leutenegger, C., Willgert, K. J. E., Brisbane-Cohen, L., Richardson-Lopez, J., Holmes, E. C. and Parrish, C. R. 2017. Spread of canine influenza A(H3N2) virus, United States. *Emerg Infect. Dis.* **23**: 1950–1957.

34. Yachi, A. and Mochizuki, M. 2006. Survey of dogs in Japan for group 2 canine coronavirus infection. *J. Clin. Microbiol.* **44**: 2615–2618.
### Table 1. Numbers (%) of dogs with multiple infection

| Signalement                  | CIRD n=61 | respiratory disease, not CIRD n=83 | asymptomatic n=23 | CIRD n=14 | asymptomatic n=66 |
|------------------------------|-----------|------------------------------------|-------------------|-----------|-------------------|
| **Gender**                   |           |                                     |                   |           |                   |
| Male                         | 20 (32.8) | 30 (36.1)                           | 1 (4.3)           | 5 (35.7) | 15 (0.2)          |
| Female                       | 8 (13.1)  | 8 (13.1)                            | 0.0 (0.0)         | 0.0 (0.0) | 0.0 (0.0)         |
| Spayed female                | 19 (31.1) | 5 (6.0)                             | 3 (13.0)          | 4 (28.6) | 22 (0.3)          |
| Unknown                      | 6 (9.8)   | 6 (9.8)                             | 1 (4.3)           | 0.0 (0.0) | 20 (0.3)          |
| **Age**                      |           |                                     |                   |           |                   |
| <6 month                     | 43 (70.5) | 3 (3.6)                             | 1 (4.3)           | 10 (71.4)| 24 (16.4)         |
| 7 month - 1 year             | 5 (8.2)   | 7 (8.4)                             | 1 (4.3)           | 1 (7.1)  | 3 (4.3)           |
| 2 - 5 year                   | 5 (8.2)   | 6 (7.2)                             | 4 (17.4)          | 1 (7.1)  | 10 (15.2)         |
| 6 - 10 year                  | 3 (4.9)   | 26 (31.3)                           | 12 (52.2)         | 1 (7.1)  | 18 (16.0)         |
| <11 year                     | 5 (8.2)   | 36 (43.4)                           | 5 (21.7)          | 1 (7.1)  | 3 (4.3)           |
| Unknown                      | 0 (0.0)   | 0 (0.0)                             | 0.0 (0.0)         | 0.0 (0.0) | 8 (0.1)           |
| **Environment**              |           |                                     |                   |           |                   |
| Indoor                       | 58 (95.1) | 74 (89.2)                           | 22 (95.7)         | 6 (42.9) | 16 (24.2)         |
| Outdoor                      | 0 (0.0)   | 1 (1.2)                             | 1 (4.3)           | 0.0 (0.0) | 1 (1.5)           |
| Unknown                      | 1 (1.6)   | 6 (7.2)                             | 0.0 (0.0)         | 3 (21.4) | 40 (60.6)         |
| **Inmate animals**           |           |                                     |                   |           |                   |
| No                           | 39 (63.9) | 18 (21.7)                           | 12 (52.2)         | 0.0 (0.0) | 0.0 (0.0)         |
| More than one animal         | 17 (27.0) | 54 (63.1)                           | 11 (47.4)         | 0.0 (0.0) | 0.0 (0.0)         |
| Unknown                      | 5 (8.2)   | 36 (43.4)                           | 5 (21.7)          | 3 (21.4) | 4 (5.5)           |
| **Vaccination**              |           |                                     |                   |           |                   |
| Vaccinated                   | 44 (72.1) | 46 (55.4)                           | 21 (91.3)         | 3 (21.4) | 11 (16.7)         |
| None                         | 2 (3.3)   | 3 (3.6)                             | 0.0 (0.0)         | 0.0 (0.0) | 0.0 (0.0)         |
| Unknown                      | 15 (24.6) | 32 (38.6)                           | 1 (4.3)           | 1 (7.1)  | 55 (83.3)         |

**CIRD**: Canine infectious respiratory disease; 95% CI: 95% Confidence interval.

**a** vaccinaiton defined as application including canine parainfluenza virus (CPiV) and canine adenovirus type-2 (CAV-2) with core vaccination.

**significantly higher than respiratory disease dogs and asymptomatic dogs (p < 0.01)**

**Table 2. Numbers (%) of dogs with multiple infection**

| CIRD n=61 | respiratory disease, not CIRD n=83 | asymptomatic n=23 | CIRD n=14 | asymptomatic n=66 |
|-----------|------------------------------------|-------------------|-----------|-------------------|
| #         | %                                 | 95% CI            |           |                   |
| Gender     |                                    |                   |           |                   |
| Male       | 20 (32.8)                          | 21.3-46.0         | 1 (4.3)   | 35.7              | 15.0 (0.2) | 13.3-34.7 |
| Female     | 8 (13.1)                           | 5.8-24.2          | 3 (13.0)  | 23.2              | 0.0 (0.0) | 0.0-22.1  |
| Spayed female | 19 (31.1)                       | 19.4-43.3         | 5 (6.0)   | 21.3              | 0.0 (0.0) | 0.0-22.1  |
| Unknown    | 6 (9.8)                            | 3.7-20.2          | 5 (6.0)   | 21.3              | 0.0 (0.0) | 0.0-22.1  |
| Age        |                                    |                   |           |                   |
| <6 month   | 43 (70.5)                          | 57.4-81.5         | 1 (4.3)   | 1-21.9            | 10 (71.4) | 41.9-91.6 |
| 7 month - 1 year | 5 (8.2)                             | 2.7-18.1          | 7 (8.4)   | 7.5               | 1 (7.1)  | 0.2-33.9  |
| 2 - 5 year | 5 (8.2)                            | 2.7-18.1          | 6 (7.2)   | 5.6               | 1 (7.1)  | 0.2-33.9  |
| 6 - 10 year| 3 (4.9)                            | 1.0-13.7          | 26 (33.3) | 31.6              | 12 (52.2)| 30.6-73.2 |
| <11 year   | 5 (8.2)                            | 2.7-18.1          | 36 (43.4)| 32.5              | 5 (21.7) | 7.5-63.7  |
| Unknown    | 0 (0.0)                            | 0.0-4.8           | 0 (0.0)   | 0.0-12.2          | 0 (0.0)  | 0.0-22.1  |
| Environment|                                    |                   |           |                   |
| Indoor     | 58 (95.1)                          | 86.3-99.0         | 74 (89.2)| 80.6-94.9         | 22 (95.7)| 78.1-99.9 |
| Outdoor    | 0 (0.0)                            | 0.0-4.8           | 1 (1.2)   | 0.6               | 1 (4.3)  | 0.1-21.9  |
| Unknown    | 1 (1.6)                            | 0.0-8.8           | 6 (7.2)   | 5.7               | 0 (0.0)  | 0.0-12.2  |
| Inmate animals|                                   |                   |           |                   |
| No         | 39 (63.9)                          | 50.6-75.8         | 18 (21.7)| 13.4              | 12 (52.2)| 30.6-73.2 |
| More than one animal | 17 (27.0)                        | 17.1-48.0         | 54 (63.1)| 33.6-75.2         | 11 (47.4)| 26.8-69.4 |
| Unknown    | 5 (8.2)                            | 2.7-18.1          | 11 (13.3)| 6.8               | 0 (0.0)  | 0.0-12.2  |
| Vaccination|                                    |                   |           |                   |
| Vaccinated | 44 (72.1)                          | 59.2-82.9         | 46 (55.4)| 44.1-66.3         | 21 (91.3)| 72-98.9 |
| None       | 2 (3.3)                            | 0.4-13.3          | 3 (3.6)   | 2.0               | 0 (0.0)  | 0.0-12.2  |
| Unknown    | 15 (24.6)                          | 14.5-37.3         | 32 (38.6)| 28.1              | 1 (4.3)  | 0.1-21.9  |

**CIRD**: Canine infectious respiratory disease; 95% CI: 95% Confidence interval.

**a** vaccinaiton defined as application including canine parainfluenza virus (CPiV) and canine adenovirus type-2 (CAV-2) with core vaccination.

**significantly higher than respiratory disease dogs and asymptomatic dogs (p < 0.01)**
Table 2. Numbers of dogs with multiple infection

| Pathogens                     | Household dogs | Kennel housed dogs |
|-------------------------------|----------------|-------------------|
|                               | n=64           | n=83              |
|                               | CIRD, respiratory disease | asymptomatic |
|                               | positive no. (%) | 95% CI | positive no. (%) | 95% CI | positive no. (%) | 95% CI |
| B. bronchiseptica             | 17 (27.9)      | 17.1-40.8        | 8 (9.6)         | 4.3-18.1 | 1 (4.3)         | 0.1-12.9 |
| CHV-1                         | 1 (1.6)        | 0.0-8.8          | 1 (1.2)         | 0.0-6.5  | 0 (0.0)         | 0.0-12.2  |
| CPiV                          | 1 (1.6)        | 0.0-8.8          | 0 (0.0)         | 0.0-3.5  | 0 (0.0)         | 0.0-12.2  |
| CPnV                          | 1 (1.6)        | 0.0-8.8          | 0 (0.0)         | 0.0-3.5  | 0 (0.0)         | 0.0-12.2  |
| B. bronchiseptica+CPiV        | 2 (4.9)        | 1.0-13.7         | 0 (0.0)         | 0.0-3.5  | 0 (0.0)         | 0.0-12.2  |
| B. bronchiseptica+CPnV        | 2 (3.3)        | 0.4-11.3         | 0 (0.0)         | 0.0-3.5  | 0 (0.0)         | 0.0-12.2  |
| B. bronchiseptica+M. cynos    | 2 (3.3)        | 0.4-11.3         | 0 (0.0)         | 0.0-3.5  | 0 (0.0)         | 0.0-12.2  |
| B. bronchiseptica+CPiV+CAV2   | 1 (1.6)        | 0.0-8.8          | 0 (0.0)         | 0.0-3.5  | 0 (0.0)         | 0.0-12.2  |
| M. cynos+CPiV                 | 1 (1.6)        | 0.0-8.8          | 0 (0.0)         | 0.0-3.5  | 0 (0.0)         | 0.0-12.2  |

**significantly higher than respiratory disease dogs and asymptomatic dogs (p<0.01)**
**Table 3. Numbers of dogs with positive individual CIRD pathogens**

| Pathogens | CIRD | respiratory disease, not CIRD | asymptomatic |
|-----------|------|-------------------------------|--------------|
|           | n=61 | n=83                          | n=23         | n=14 | n=66 |
| B. bronchiseptica | 27 (44.3) | 8 (9.6) | 1 (4.3) | 4 (28.6) | 11 (16.7) |
| CAV-2     | 1 (1.6) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| CDV       | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| CHV-1     | 5 (8.2) | 1 (1.2) | 0 (0.0) | 0 (0.0) | 2 (3.0) |
| CIV       | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| CPoV      | 3 (4.9) | 1 (1.6) | 0 (0.0) | 0 (0.0) | 4 (6.1) |
| CPnV      | 3 (4.9) | 1 (1.6) | 0 (0.0) | 0 (0.0) | 1 (1.5) |
| CRCoV     | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| M. cynos  | 2 (4.9) | 1.0-13.7 | 0 (0.0) | 0.0 (0.0) | 1.0-13.7 |

CIRD, Canine infectious respiratory disease; 95% CI, 95% Confidence interval; CAV-2, Canine adenovirus type-2; CDV, Canine distemper virus; CHV-1, Canine herpesvirus type 1; CIV, Canine influenza virus; CPoV, Canine parainfluenza virus, CPnV, Canine pneumovirus; CRCoV, Canine respiratory coronavirus

**significantly higher than asymptomatic dogs (p<0.01)**