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LETTER

Co-circulation of H3N2 canine influenza and distemper viruses in canine populations from Liaoning, China

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Highlights

- Co-circulation and high-frequency co-infection (up to 66.67%) of H3N2 canine influenza virus and canine distemper virus were first reported in dog population, with no significant genetic variation in CIV observed during co-infection.
- The canine distemper epidemic was a key driver of the sharp increase in canine influenza virus prevalence, with statistical analysis revealing a significant positive correlation that may be primarily attributed to CDV-induced immunosuppression.

Influenza A virus (IAV) has a wide host range, including wild birds, poultry, various mammals, and even humans (Xu *et al.* 2024). Currently, two subtypes of canine influenza virus (CIV), H3N8 and H3N2, are primarily circulating in dogs. The H3N8 CIV was introduced from horses into dogs in 2004 (Crawford *et al.* 2005), while the H3N2 CIV originated from chickens in Asia in 2007 (Song *et al.* 2008). In China, H3N2 is the predominant CIV subtype, with a prevalence rate of up to 5.63% in the canine population, as reported by Chen *et al.* (2023). CIV infection typically manifests with symptoms such as coughing, sneezing, runny nose, and fever but is rarely fatal. However, co-infection with other pathogens (e.g., *Streptococcus*, *Mycoplasma* or canine parainfluenza virus) can exacerbate symptoms and lead to lethal outcomes (Yondo *et al.* 2023).

Dogs, as companion animals, are in close contact with humans and can serve as “mixing vessels” for influenza viruses, as they can be infected by both human- and avian-origin IAV (Ning *et al.* 2012). Emerging reassortants between H3N2 CIV and human-origin 2009/H1N1 virus, avian-origin H9N2, H5N1, or swine-origin H1N1 viruses have been detected in dogs (Na *et al.* 2015; Lee *et al.* 2016; Wang *et al.* 2019). The increasing number of pet dogs in China raises concerns about the potential public health threat posed by CIV. Therefore, it is crucial to monitor the epidemic situation and zoonotic risks associated with CIVs.

Liaoning Province hosts the largest pet dog breeding and trading market in China. Annually, nearly five million pet dogs and cats are transported from Liaoning to other provinces (data sourced from Northeast News Network; http://liaoning.nen.com.cn/network/liaoningnews/lnnewsjingji/2024/03/29/628135240103105444.shtml). Consequently, epidemiological surveillance of canine and feline pathogens is essential for the early warning of infectious diseases in China. Since 2018, we have conducted CIV surveillance in an animal shelter in Liaoning, performing random surveillance at least twice annually. PCR based on the IAV matrix (M) gene was conducted for the identification of CIV infections (the PCR primers and methods are available upon request). From 2018 to 2022, twelve surveillance activities were conducted, with CIV infections detected in six instances. The overall positive rate for the entire surveillance period was 6.18%, with positive rates of 11.04, 10.45, 11.81, 2.50, 10.00, and 1.45% for the six detections, respectively. Eight H3N2 CIVs, seven H3N6 reassortants (Meng *et al.* 2023), and one H9N2 avian-origin virus were identified from these positive samples, with the H3N6 and H9N2 virus detected on 8/30/2019 and 3/11/2022, respectively.

An H3N2 CIV outbreak occurred among these dogs starting in April 2023. Unusually high CIV positive rates were detected in surveillance activities on 4/26/2023, 7/22/2023, and 10/11/2023, with rates of 70.83, 26.67, and 31.82%, respectively. While the infection rate of canine influenza was surging dramatically, canine distemper was also identified

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as an emerging epidemic among canine populations. The positive rate of canine distemper virus (CDV) ranged from 16.67 (9/29/2023) to 79.17% (4/26/2023). This CDV epidemic persisted for nearly 14 mon, with no CDV-positive dogs detected after 6/9/2024. As the canine distemper outbreak subsided, the infection rate of canine influenza returned to pre-epidemic levels. CIV and CDV co-infections were found in four surveillances, with the highest co-infection rate reaching 66.67% (16/24, 95% CI: 44.70–83.58%) (Fig. 1-A). After inquiry, the sampled dog population had not been vaccinated against any diseases, including canine influenza and canine distemper.

Forty H3N2 CIV strains were isolated during the outbreak. The whole genomes of 16 CIV strains isolated from co-infection samples were compared with CIV strains isolated from our surveillance and representative strains from other provinces in China. All CIV strains HA genes in our study exhibited high sequence identity (97.8–100%) with other Liaoning isolates. As shown in Fig. 1-B, the HA genes from co-infection CIV strains clustered with other Liaoning CIV isolates. The H genes of co-infection CDV strains showed 98.0–100% identity to circulating strains. Phylogenetically, these CDV strains clustered within the Asia-1 lineage but were distinct from the vaccine strain, America-1 strain (Fig. 1-C). Furthermore, we compared the molecular markers associated with transmissibility among CIV strains: those infected alone during the epidemic (A/canine/Liaoning/SY04105/2023(H3N2), A/canine/Liaoning/SY10302/2023(H3N2), and A/canine/Liaoning/SY10305/2023(H3N2)), those co-infected with CDV during

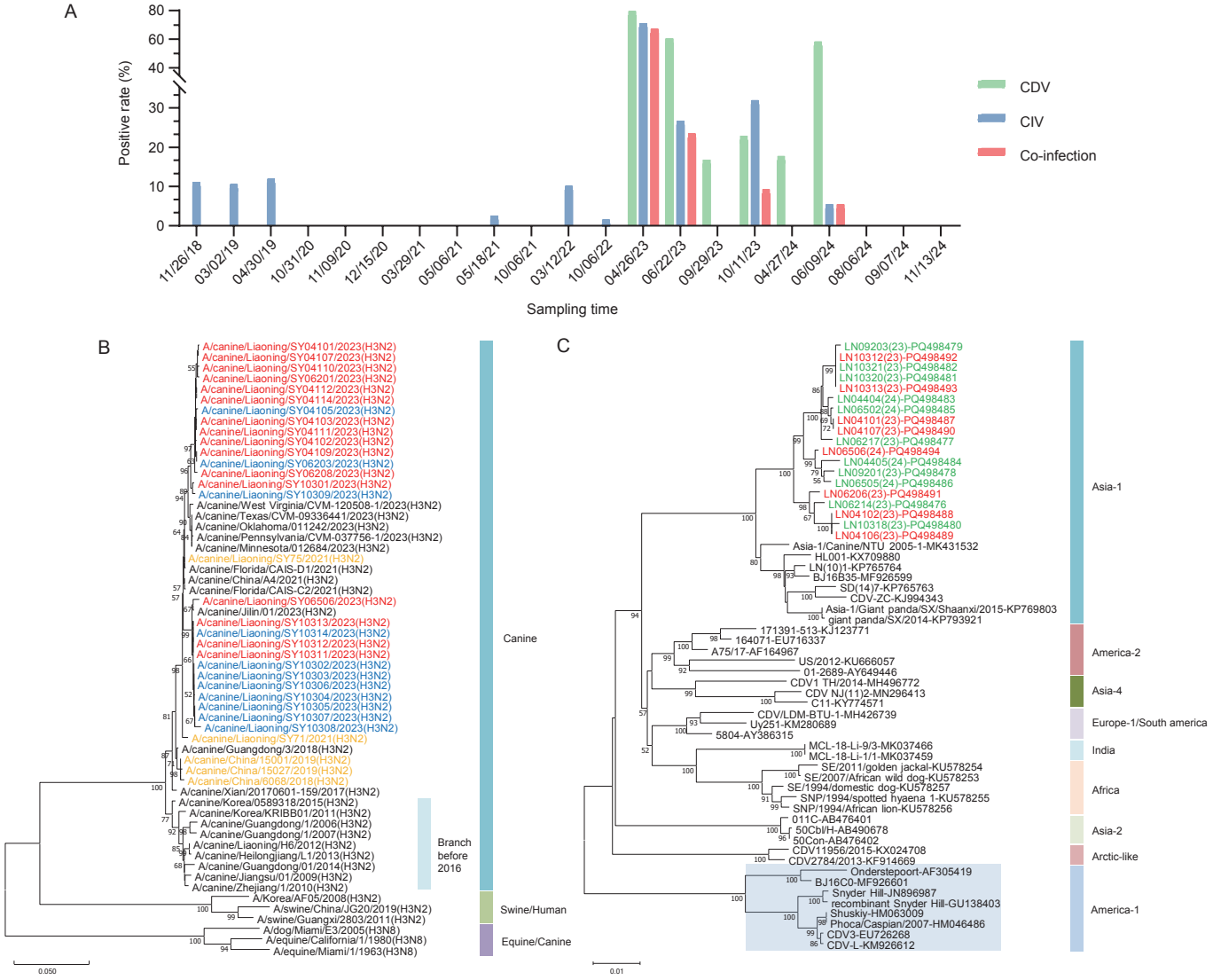


Fig. 1 Epidemiological surveillance and phylogenetic analysis of canine infuenza virus (CIV) and canine distemper virus (CDV) in dogs. A, positive rates of CIV, CDV, and co-infections detected by RT-PCR from 2018 to 2024. B, phylogenetic analysis of the HA gene of CIVs using the neighbor-joining method with 1,000 bootstrap replicates (MEGA 11). The sequence name with color (blue, red, yellow) were the viruses identified in this study, and the sequences with black were download from the database. Blue indicated CIV strains detected as single infections during the epidemic, red indicated CIV strains co-infected with CDV during the epidemic, and yellow indicated CIV strains identified prior to the epidemic. C, phylogenetic analysis of the H gene of CDVs using the neighbor-joining method with 1,000 bootstrap replicates (MEGA 11). Representative CDV vaccines were highlighted in blue. The sequence name with color (green, red) were the viruses identified in this study, and the sequences with black were download from the database. Green indicated CDV strains detected as single infections, and red indicated CDV strains co-infected with CIV.

the epidemic (A/canine/Liaoning/SY04101/2023(H3N2), A/canine/Liaoning/SY04103/2023(H3N2), A/canine/Liaoning/SY04110/2023(H3N2), and A/canine/Liaoning/SY06201/2023(H3N2)), and those identified prior to the epidemic (A/canine/China/6068/2018(H3N2), A/canine/China/15001/2019(H3N2), A/canine/China/15027/2019(H3N2), A/canine/Liaoning/SY71/2021(H3N2), and A/canine/Liaoning/SY75/2021(H3N2)). As shown in Appendix A, no significant differences were observed in functional amino acid mutations contributing to enhanced transmissibility among the tested strains.

Given that the genetic and molecular markers associated with CIV transmissibility did not significantly differ from those of previous strains, we hypothesized that the high prevalence of CIV infection was related to the prevalence of CDV. To further clarify the impact of the CDV epidemic on CIV prevalence, we conducted a retrospective study. Using the PCR method, we detected the presence of CDV and other common canine respiratory viruses, including canine parainfluenza virus (CPIV), canine respiratory coronavirus (CRCoV), and canine adenovirus type 2 (CAV-2), in all 680 dog samples collected between 2020 and 2024. No CDV was detected before or after its epidemic, and neither CRCoV nor CAV-2 was detected in the dog population. CPIV was detected in three surveillance activities (10/6/2022, 9/29/20023 and 10/11/2023), with positive rates of 1.45, 11.11, and 2.27%, respectively (Appendix B). Multiple linear regression analysis was used to assess the association between CIV prevalence and the prevalence of CDV and CPIV (continuous variables). The forced entry method was employed for all examined variables (potential confounders) in the multivariate model. The estimates, including adjusted linear regression β coefficients, standard error (SE), t-value, and P-value, were calculated. Goodness-of-fit was assessed by estimating the adjusted coefficient of determination (R^2), F-value, and variance inflation factor (VIF) (Li *et al.* 2023). As shown in Appendix C, the analysis revealed that CIV prevalence was significantly and positively correlated with CDV positivity (P<0.001), but the presence of CPIV did not affect the CIV infection rate in the dog population (P=0.628).

CDV is a fatal pathogen for both domestic and wild animals, infecting various Carnivora, Canidae, Mustelidae, Procyonidae, and Felidae. CDV is highly contagious, with infection rates in dogs in China ranging from 9.4 to 45% (Hao *et al.* 2019; Zhou *et al.* 2024). CDV infects myeloid cells and disseminates via circulating lymphocytes, leading to host immune suppression (Laksono *et al.* 2023). In this state, the host becomes highly vulnerable to other pathogens, increasing morbidity and mortality. Co-infections with CDV and other pathogens had been found in multiple hosts (Munson *et al.* 2008). This study is the first to report the co-infection with CDV and CIV. It is important to recognize that an outbreak of an animal infectious disease may increase the prevalence of another zoonotic pathogen. The prevalence of H5N1 virus in North American dairy cattle is also likely related to diseases associated with immunosuppression prevalent in the herd, underscoring the need to test for other pathogens in related studies.

In summary, this study reported an outbreak of CIV and CDV co-infections in an animal shelter in Liaoning Province, China 2023, demonstrating that co-infection with CDV increased the prevalence of CIV. Additionally, co-infection did not affect the genetic evolution patterns of these two viruses. We hypothesized that CDV-induced immunosuppression was the primary reason for the increased prevalence of CIV. This study suggested that vaccinations are necessary and provided further insights into the molecular epidemiology of CIV and CDV.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used DeepSeek to assist in improving the clarity and readability of the language. All content generated was carefully reviewed and revised by the authors as necessary, and the authors take full responsibility for the final content of the article.

Ethical statements

The study has no ethical implications.

Data availability

The whole genome or the HA gene of CIVs was sequenced and have been deposited in GenBank under the following accession numbers: PV276838-PV276857, PV290969-PV291040; the H gene of CDVs was sequenced and have been deposited in GenBank under the following accession numbers: PQ498476-PQ498494.

Appendices are associated with this study are available at https://doi.org/10.1016/j.jia.2025.08.006

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