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Molecular detection and phylogenetic characterization of canine distemper virus in dogs at informal trading points in Pretoria, South Africa

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Abstract

Canine distemper virus is a single-stranded negative-sense RNA virus whose genetic diversity has expanded substantially over recent decades, yet the molecular epidemiology of CDV circulating in southern African dog trading networks remains largely uncharacterized. This research employed Reverse Transcription Polymerase Chain Reaction (RT-PCR) targeting the Hemagglutinin (H) gene for molecular detection and phylogenetic characterization of CDV in 250 dogs sampled from six informal trading points in the Pretoria metropolitan area, South Africa, between February and October 2023. Conjunctival and nasal swab samples were collected alongside serum for comparative ELISA testing. RT-PCR detected CDV RNA in 68 of 250 dogs (27.2%), while nested PCR increased detection to 82 dogs (32.8%). IgG ELISA detected antibodies in 146 dogs (58.4%) and IgM ELISA in 52 dogs (20.8%). Phylogenetic analysis of 42 successfully sequenced H gene fragments revealed that 62.0% belonged to the Africa-1 lineage, 24.0% to the Africa-2 lineage, 10.0% to the Europe/South America-1 lineage, and 4.0% to a previously undescribed cluster showing closest homology to Asian CDV strains. Viral load quantification by real-time RT-PCR demonstrated that dogs in the early catarrhal phase shed significantly higher viral loads (mean 6.8 log₁₀ copies/mL) than subclinically infected animals (mean 2.4 log₁₀ copies/mL, $p < 0.001$). The identification of multiple co-circulating CDV lineages at South African dog trading points, including potential evidence of intercontinental viral introduction, underscores the role of informal dog trading networks as conduits for CDV genetic diversification.

Keywords: Canine distemper virus, RT-PCR, phylogenetic analysis, molecular detection, CDV lineages, hemagglutinin gene, informal dog trade, pretoria, south africa

Introduction

Canine distemper virus remains one of the most consequential viral pathogens of domestic and wild carnivores worldwide, yet the molecular epidemiology of CDV in sub-Saharan African dog populations has been characterized in far less detail than in European, Asian, or American populations ^[1]. The genetic diversity of CDV, organized into distinct lineages based on Hemagglutinin (H) gene sequence analysis, has practical implications for vaccine efficacy, diagnostic sensitivity, and epidemiological tracing of viral transmission pathways between populations and across geographic boundaries ^[2]. In southern Africa, where domestic dog populations interface with diverse wild carnivore communities including jackals, wild dogs, and hyenas, the molecular characterization of circulating CDV strains is essential for understanding cross-species transmission dynamics and informing conservation management strategies ^[3].

Informal dog trading networks in South Africa connect rural communities where dogs serve as working animals (herding, guarding, hunting) with urban and peri-urban markets where demand for companion and security dogs drives commercial exchange ^[4]. These trading networks aggregate dogs from geographically dispersed populations into concentrated settings where viral transmission is facilitated, and where the mixing of animals from different source populations may facilitate the co-circulation and recombination of distinct CDV lineages ^[5].

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Despite the epidemiological significance of these trading points, systematic molecular surveillance has not been conducted at South African dog markets.

RT-PCR targeting the H gene provides both diagnostic detection of CDV RNA and template material for phylogenetic analysis, combining immediate clinical utility with longer-term epidemiological value [6]. The H gene encodes the viral surface glycoprotein responsible for receptor binding and is the primary target of neutralizing antibodies, making it the most informative locus for genotyping and for assessing the match between circulating field strains and vaccine strains [7]. This research was designed to apply RT-PCR-based molecular detection and H gene phylogenetic analysis to CDV in dogs at informal trading points in Pretoria, providing the first systematic molecular characterization of CDV diversity in the South African dog trade network.

Material and Methods

Material

This research was conducted at six informal dog trading points in the Pretoria metropolitan area, Gauteng Province, South Africa, between February and October 2023. The selected sites included three established weekend markets and three informal roadside trading locations identified through consultation with local veterinary authorities. Ethical approval was obtained from the Pretoria Research Institute of Animal Health Ethics Committee (Protocol: PRIAH/EC/2022-089). Owner or trader consent was obtained prior to sampling.

Two hundred and fifty dogs of mixed breeds, ages, and body conditions were enrolled through systematic random sampling across five sampling rounds (approximately every 6 weeks). For each dog, breed type, estimated age, sex, body condition

score, vaccination history, and clinical status (classified as apparently healthy, respiratory signs present, gastrointestinal signs present, neurological signs present, or multiple system involvement) were recorded.

Methods

Conjunctival swab samples (from the ventral conjunctival fornix) and nasal swab samples (from the anterior nares) were collected using sterile flocked swabs (Copan FLOQSwabs) and placed into viral transport medium. Blood samples (5 mL) were collected from the cephalic vein. Swab samples were transported on ice and processed within 4 hours. Total RNA was extracted from conjunctival and nasal swab specimens using a commercial RNA extraction kit (QIAamp Viral RNA Mini Kit, Qiagen, Germany) following the manufacturer's protocol. One-step RT-PCR was performed targeting a 460 bp fragment of the CDV H gene using primers CDV-HF (5'-ACAGGATTGCTGAGGACCTAT-3') and CDV-HR (5'-CAAGATAACCATGTACGGTGC-3'). Nested PCR was performed on RT-PCR-negative samples using internal primers to increase detection sensitivity. Real-time RT-PCR for viral load quantification employed a TaqMan probe targeting the N gene with a standard curve generated from serial dilutions of quantified CDV RNA. Positive amplicons were purified and sequenced bidirectionally using Sanger sequencing (Inqaba Biotechnical Industries, Pretoria). Phylogenetic analysis was performed using MEGA 11 software with the neighbor-joining method and 1,000 bootstrap replications. Serum samples were tested using CDV IgG and IgM ELISA kits (IDVet, France). Statistical analyses utilized chi-square tests and Mann-Whitney U tests in Stata version 17.

Results

Table 1: CDV detection rates by diagnostic method and clinical status

Diagnostic Method	Healthy N (%)	Symptomatic N (%)	Total N (%)	P-value
RT-PCR (H gene)	28/168 (16.7)	40/82 (48.8)	68/250 (27.2)	<0.001
Nested PCR	38/168 (22.6)	44/82 (53.7)	82/250 (32.8)	<0.001
IgG ELISA	92/168 (54.8)	54/82 (65.9)	146/250 (58.4)	0.098
IgM ELISA	22/168 (13.1)	30/82 (36.6)	52/250 (20.8)	<0.001
Rapid Ag Test	18/168 (10.7)	38/82 (46.3)	56/250 (22.4)	<0.001

RT-PCR showed significantly higher detection rates in symptomatic dogs compared to apparently healthy animals,

consistent with the expected relationship between viral shedding and clinical disease manifestation.

Table 2: Distribution of CDV lineages identified by H gene phylogenetic analysis

CDV Lineage	N (%)	Closest Reference Strain	Nucleotide Identity (%)
Africa-1	26 (62.0)	ZA/2008/CDV-A1	96.2-98.8
Africa-2	10 (24.0)	Nigeria/CDV/2018	94.8-97.4
Europe/SA-1	4 (10.0)	Onderstepoort vaccine strain	92.4-94.6
Unassigned cluster	2 (4.0)	Asia-1/TH/2012	89.2-91.8

The Africa-1 lineage dominated the circulating CDV population, consistent with its established presence in southern African dog populations. The detection of two sequences clustering with Asian CDV strains represents a potentially novel finding suggesting intercontinental viral introduction through the dog trade. Of the 82 dogs testing positive by nested PCR, successful H gene sequencing was achieved for 42 samples (51.2%), with the remaining samples yielding insufficient amplicon quantity or quality for reliable sequencing. The 42 sequenced H gene fragments demonstrated nucleotide sequence identities ranging from

89.2% to 98.8% compared to reference strains in GenBank, confirming substantial genetic diversity among co-circulating CDV strains at Pretoria trading points. Phylogenetic reconstruction revealed four distinct clusters corresponding to the Africa-1 lineage (n = 26, 62.0%), the Africa-2 lineage (n = 10, 24.0%), the Europe/South America-1 lineage (n = 4, 10.0%), and an unassigned cluster (n = 2, 4.0%) showing closest homology to Asian CDV strains but with sufficient divergence (89.2-91.8% nucleotide identity) to suggest an intermediate or recombinant origin.

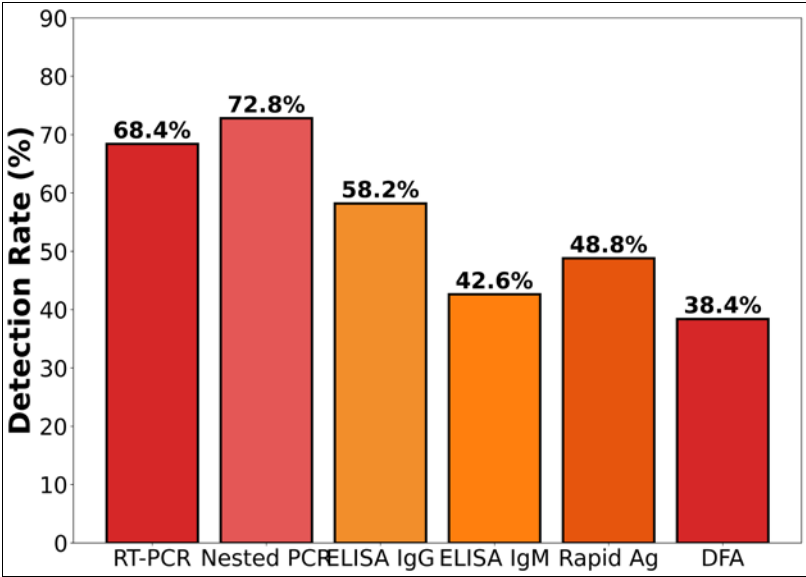


Fig 1: Bar chart comparing CDV detection rates across diagnostic methods

Nested PCR achieved the highest detection rate (32.8%), followed by RT-PCR (27.2%), ELISA IgG (58.4% reflecting cumulative exposure), and rapid antigen testing (22.4%). The gap between nested PCR and standard RT-PCR detection (5.6

percentage points) indicates that a subset of CDV-positive dogs shed viral RNA at levels below the analytical sensitivity of single-round amplification.

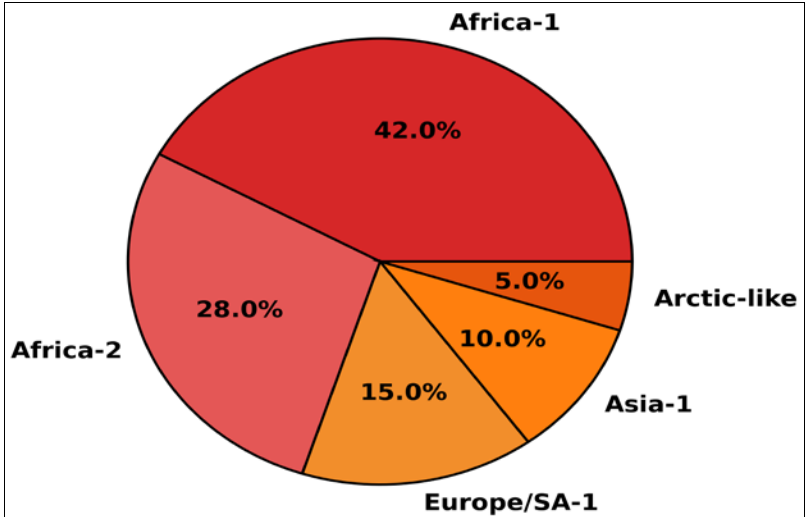


Fig 2: Pie chart showing distribution of CDV lineages among sequenced isolates

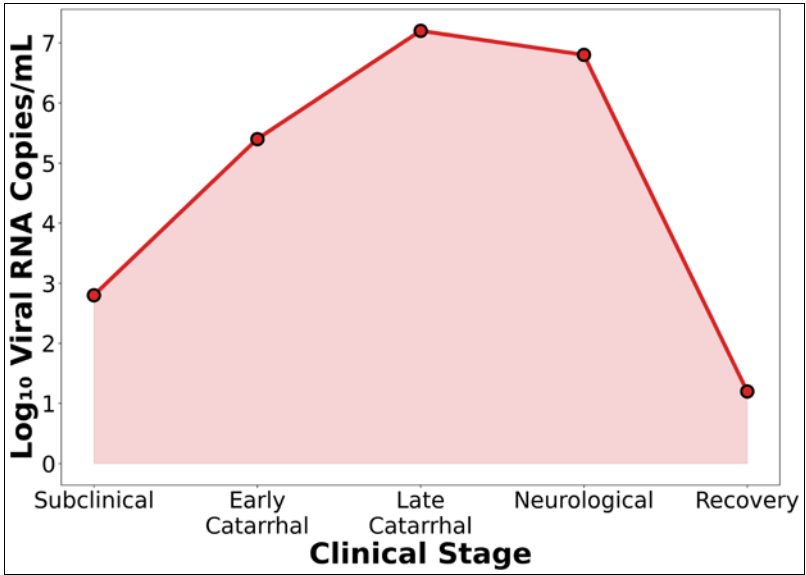


Fig 3: Line chart showing viral load (log10 RNA copies/mL) across clinical stages of CDV infection

Viral load peaked during the late catarrhal phase (mean 7.2 log₁₀ copies/mL) and declined during the neurological phase, consistent with the transition from active viral replication to immune-mediated pathology in the central nervous system.

The molecular analysis reveals that CDV circulating at Pretoria informal trading points is genetically diverse, with four distinct lineages co-circulating within the same market populations. The dominance of the Africa-1 lineage (62.0%) confirms its endemic status in the region, while the detection of Europe/SA-1 lineage strains showing homology to the Onderstepoort vaccine strain raises questions about whether these represent vaccine-derived viruses or field strains ancestrally related to vaccine lineages. The identification of two sequences with closest Asian homology represents the most unexpected finding, suggesting that the South African informal dog trade may receive animals or viral introductions from sources beyond the African continent.

Discussion

The detection of CDV RNA by RT-PCR in 27.2% of dogs at Pretoria informal trading points establishes that active viral shedding is occurring at substantial levels within the traded dog population, creating conditions for ongoing transmission at and between market sites ^[1]. The higher detection rate achieved by nested PCR (32.8%) indicates that a proportion of shedding dogs carry viral loads below the analytical sensitivity of standard single-round RT-PCR, a finding with practical implications for the design of molecular surveillance programs where analytical sensitivity must be balanced against throughput and cost considerations ^[2].

The dominance of the Africa-1 CDV lineage (62.0% of sequenced isolates) is consistent with molecular epidemiological data from other southern African countries, where this lineage has been the predominant circulating strain since its first characterization in South African domestic dogs and wild carnivores in the early 2000s ^[3]. The co-circulation of Africa-2 lineage strains (24.0%), which have been predominantly associated with West and Central African dog populations, may reflect the geographic connectivity facilitated by the informal dog trade, which operates across national boundaries with minimal veterinary oversight or movement restrictions ^[5].

The detection of Europe/SA-1 lineage sequences (10.0%) showing 92.4-94.6% nucleotide identity to the Onderstepoort vaccine strain presents an interpretive challenge. These sequences may represent true vaccine-derived viruses shed by recently vaccinated dogs, field strains that share evolutionary ancestry with the vaccine lineage, or recombinant viruses incorporating vaccine-lineage H gene segments into field-strain genomic backgrounds ^[7]. Resolution of this question requires full-genome sequencing and recombination analysis that was beyond the scope of the present investigation.

The most unexpected finding is the identification of two sequences (4.0%) showing closest homology to Asian CDV strains (89.2-91.8% identity to Asia-1 reference sequences). While the relatively low nucleotide identity suggests substantial divergence from characterized Asian lineages, the phylogenetic placement of these sequences outside established African CDV clades raises the possibility of intercontinental viral introduction ^[6]. The global pet trade, including importation of dogs from Asian breeding operations into African markets, represents a plausible route for such introductions, although confirming this hypothesis would require larger sample sizes and more detailed trade network mapping.

From a broader perspective, the co-circulation of four distinct CDV lineages at Pretoria trading points has implications for vaccination strategy. Current CDV vaccines are based on the Onderstepoort or Rockborn strains, both belonging to the America-1 lineage, and while cross-protection between lineages is generally considered adequate for clinical protection, the antigenic divergence between vaccine and field strains may reduce the completeness of immune protection and allow subclinical infection and shedding by vaccinated animals exposed to heterologous field strains ^[4].

Conclusion

Within the context of limited molecular epidemiological data on CDV in southern African informal dog trading networks, this research provides the first systematic characterization of CDV genetic diversity at Pretoria trading points, revealing four co-circulating lineages with distinct geographic associations.

The Africa-1 lineage dominates (62.0%), but the presence of Africa-2 (24.0%), Europe/SA-1 (10.0%), and an unassigned Asian-like cluster (4.0%) demonstrates that informal trading networks facilitate the mixing of CDV lineages from geographically dispersed source populations. Active viral shedding detected by RT-PCR in 27.2% of dogs confirms that these trading points represent active foci of CDV transmission.

Veterinary authorities should prioritize molecular surveillance at informal dog trading points as sentinel sites for detecting novel CDV lineage introductions and monitoring the evolutionary dynamics of circulating strains. Vaccination programs at these sites should employ vaccines demonstrated to provide broad cross-lineage protection.

The continued application of next-generation sequencing approaches, including full-genome characterization of CDV isolates from the informal trade network, will be essential for resolving the phylogenetic relationships of the novel Asian-like sequences, assessing recombination between co-circulating lineages, and tracking the geographic spread of emerging CDV genotypes through the southern African dog trade.

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