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# Genetic characterization of canine enteric coronaviruses (CECOV-IIa) from dogs in Mosul city

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**Abstract**

Canine enteric coronavirus (CCoV) causes significant gastrointestinal illness in dogs, especially puppies, leading to high morbidity and mortality. This study investigated the presence, genetic characteristics, and clinical impact of enteric canine coronavirus (CCoV) among 50 dogs of various breeds, sexes, and age groups from different locations in Nineveh Governorate, Iraq, from August 2024 to January 2025. Clinically, infected dogs exhibited symptoms including diarrhea, vomiting, weakness, loss of appetite, dehydration, and fever. Rectal swabs were collected, and nucleic acid was extracted for virus detection using RT-PCR, which yielded clear positive bands at an amplicon size of 684 base pairs. The overall infection rate for CCoV by RT-PCR was 54% (27/50). Stray dogs demonstrated a higher infection rate compared to owned dogs. Female dogs were significantly more likely to be infected than Males, and a significantly higher prevalence was observed in dogs younger than one year. Large breeds had a greater rate of infection, and native breeds were more frequently infected than imported breeds. Molecular analysis focused on the SIIa gene and confirmed the circulation of specific CCoV-IIa genotypes in Mosul, Iraq. Out of all samples examined, five viral isolates were successfully sequenced and deposited in GenBank. Sequence alignment analysis between local isolates and GenBank reference sequences demonstrated similarity rates ranging from 93.08% to 100%. This study provides the first detailed report of CCoV detection and genotyping in Mosul, Iraq, highlighting the virus's genetic diversity, epidemiological characteristics, and clinical significance in the region.

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## Introduction

Coronaviruses (CoVs) are a large family of enveloped, positive-sense single-stranded RNA viruses known for infecting a wide range of vertebrates, including humans, livestock, birds, bats, mice, and many other wild animals (1). They are capable of causing diverse clinical syndromes ranging from respiratory and gastrointestinal to hepatic and neurological diseases. Within this family, the canine enteric coronavirus (CECoV), belonging to the Alphacoronavirus genus (2), with a genome size ranging from approximately 16 to 31 kb (3). CECoV primarily targets the small intestinal epithelium of dogs. The resultant disease typically manifests as enteritis, with symptoms such as diarrhea, vomiting, dehydration, and, in some cases, systemic illness (4) particularly in young or susceptible dogs. Initially thought to represent a single genotype, CECoV strains are now divided into two primary genotypes: CCoV type I (CCoV-I) and CCoV type II (CCoV-II). Further categorization of CCoV-II is based on variations in the spike (S) gene, resulting in two main subtypes: CCoV-IIa: Classical enteric strains and CCoV-IIb: Recombinant strains closely related to transmissible gastroenteritis virus (TGEV).

These subtypes differ genetically, especially in the spike protein gene, and exhibit distinct tissue distributions and pathogenic characteristics (5,6). In most cases, CECoV infection leads to a mild, self-limiting diarrheal illness in young dogs (7), especially puppies. However, recent years have seen the emergence of more virulent "pantropic" strains capable of causing severe systemic disease, elevated morbidity, and occasionally high mortality rates, particularly in puppies less than five months of age (8,9). Common clinical signs include soft or watery diarrhea, vomiting, anorexia, and dehydration (10,11). More severe forms may present with hemorrhagic gastroenteritis, especially in co-infections with other viruses such as canine parvovirus 2 (CPV-2) or canine adenovirus 1 (12). Recently, certain virulent strains can result in fatal systemic infections even in the absence of co-pathogens, producing a spectrum of symptoms that may include lethargy, hemorrhagic diarrhea, ataxia, and seizures (13). Deaths due to diarrheal diseases are rare but can occur in puppies, primarily as a result of electrolytes and water loss leading to dehydration, acidosis, and shock (14).

The primary route of CECoV transmission is fecal-oral, often through contact with contaminated food, water, objects, or environments. Infected dogs typically excrete the virus in feces for 6 to 9 days, though prolonged shedding for months can occur (12). This facilitates persistent environmental contamination and ongoing transmission, especially in situations of crowding such as kennels, where outbreaks can approach 100% morbidityCECoV infection prevalence is influenced by age, population density, and environmental conditions, with higher rates observed in young puppies (18) and kennel environments (13). Notably, asymptomatic dogs may serve as silent carriers (14), shedding the virus without overt clinical disease, fueling unnoticed spread within populations. Given the overlap of clinical symptoms between CECoV and other enteric pathogens (e.g., canine rotavirus, CPV-2, and canine adenovirus), laboratory confirmation is crucial for accurate diagnosis.

Coronaviruses possess some of the largest RNA genomes (12) among animal viruses, contributing to their propensity for genetic variation. Such diversity manifests in distinct strains and genotypes, with ongoing concerns about recombination and emergence of new, potentially more pathogenic variants.

To date, no comprehensive genetic characterization of CECoV has been reported in Mosul, Iraq. Given the likelihood of locally distinct genetic variants and possible recombinant forms, this study seeks to isolate and genetically characterize CECoV strains from local dogs. It further aims to explore transmission patterns shaped by local demographic and environmental conditions, and to assess whether novel recombination events have influenced the pathogenicity or adaptation of the virus within the regional canine population.

## Materials and methods

### Ethical approval

The Scientific Committee of the College of Veterinary Medicine at the University of Mosul's Department of Microbiology (Approval ID: UM.VET 2024.033, dated 09/07/2024) examined and approved this work. Samples were collected from animals and handled with the utmost care, following all accepted scientific and ethical guidelines.

### Animals

A total of 50 dogs from various regions of Nineveh Governorate, Iraq, were investigated for the presence of Enteric Canine Coronavirus (CECoV) between August 2024 and January 2025. The sampled population included dogs of different ages, sexes, and breeds (15).

### Samples collection

For each animal, a questionnaire was completed by the owner, recording details such as sex, age, breed, source, housing conditions (group or individual), and vaccination history. Rectal swabs were collected from each dog, added to a sterile tube containing 2ml of viral transport media, and then placed in a cooled box with ice packs and promptly transported to the laboratory for further processing or stored at -20°C until used (16,17).

### RNA Extraction for conventional RT-PCR

Total RNA was extracted from the collected rectal or fecal swabs utilizing the QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The concentration and purity of the extracted RNA were assessed employing a NanoDrop 2000/2000 spectrophotometer (Thermo Fisher Scientific Inc., USA). RNA concentrations ranged from 20 to 80 ng/μL, with purity (A260/280 ratio) between 1.8 and 2.0. After extraction, the RNA was either utilized right away for cDNA synthesis or kept at -20 °C for further processing.

### cDNA synthesis

Complementary DNA (cDNA) was synthesized from RNA using a commercial RT-PCR kit (GDSBio, China), following the manufacturer's instructions. Reverse transcription was carried out with the supplied reverse transcriptase and the specific primers listed in Table 1. Molecular genotyping and subtyping of canine enteric coronavirus (CECoV) S gene was performed by conventional PCR as described by (18).

**PCR amplification**

PCR amplification was performed in a 20 µL reaction mixture containing PCR Master Mix reagents as detailed in Table 2. The amplification protocol followed the conditions described in Table 3. Thermal cycling was carried out using a Mastercycler Gradient thermocycler (TECHNE, UK).

Table 1: Nucleotide Sequence of Primers

| Primer | Sequence (5′–3′) | Position | CCoVIIa Type (gene) | Amplicon size (bp) | Reference |
|---|---|---|---|---|---|
| S5F | T GCATTTGTGTCTCAGACTT | 3486–3505 | IIa (S) | 694 | (18) |
| S6R | CCAAGGCCATTTTACATAAG | 4160–4179 | | | |

Table 2: Reagents Used in Conventional RT-PCR

| Reagents | Volume (ul) |
|---|---|
| DNase/RNase-free dH $_2$ O | 13.5 |
| 10× PCR buffer | 2.5 |
| dXTP (dATP, dTTP, dCTP e dGTP) 2.5 mM | 2 |
| MgCl 2 50 mM | 0.75 |
| Forward Primer 20 pmol | 0.5 |
| Reverse Primer 20 pmol | 0.5 |
| Taq DNA polymerase (5 U/µL) | 0.25 |
| Total | 20 |

Table 3: The reaction parameters for CECoV- IIa

| PCR conditions | Temperature $^{0\,°C}$ (time) | Cycle |
|---|---|---|
| Initial denaturation | 94 (5 min) | 1 |
| Denaturation | 94 (30 sec) | |
| Annealing | 50 (45 sec) | 35 |
| Extension | 72 (1 min) | |
| Final extensions | 72 (10 min) | 1 |

Amplified PCR products were electrophoresed on an agarose gel prepared with TBE buffer for approximately 90 minutes at 80 V. DNA fragments were visualized under UV transillumination after staining with a safe DNA dye. A 100-basepair DNA ladder was utilized as a molecular size marker (19).

**DNA sequencing and submission to GenBank**

The PCR products from positive samples were sequenced using the Sanger sequencing method, with sequencing services performed by Macrogen, Korea. Briefly, the appropriate primers were mixed with 25 µL of the PCR amplicon before sequencing. The resulting sequences were saved as FASTA-format text files. Selected viral strains were submitted to the NCBI GenBank database for registration, where they were assigned unique accession numbers.

**Phylogenetic analysis**

Phylogenetic relationships among the canine coronavirus strains were analyzed using MEGA X software. The selected nucleotide sequences were aligned, and phylogenetic trees were constructed to assess genetic relatedness and evolutionary patterns.

**Statistical analysis**

Statistical analysis was conducted using IBM SPSS version 19 (20).

**Results**

Viral nucleic acid extracted from rectal swab samples was analyzed using reverse transcription polymerase chain reaction (RT-PCR) with primers specific for the S (IIa) gene. As illustrated in Figure 1, distinct positive bands were observed corresponding to the expected amplicon size of 684 base pairs.

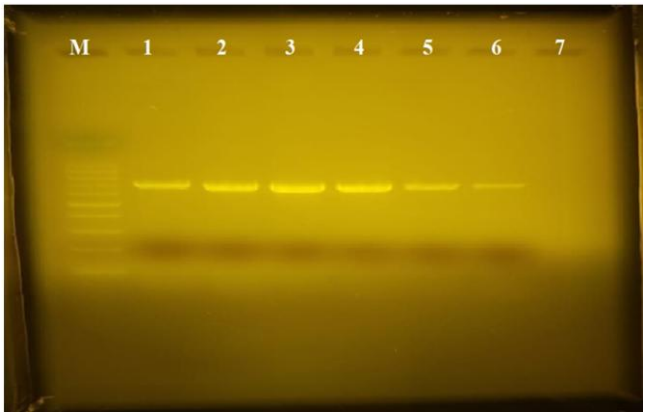


Figure 1: illustrates the gel electrophoresis image that demonstrates the PCR products. Lane (M): DNA Ladder; Lanes (1–6): Positive Samples for Canine Coronavirus (CCV); Lane (7): Negative Control. Band Size Anticipated: Approximately 684 bp.

The overall CECoV infection rate detected by PCR was 54%, with 27 out of 50 rectal swab samples testing positive. Stray dogs exhibited higher infection rates than domestic dogs, although this difference was not statistically significant. Conversely, female dogs showed significantly higher infection rates compared to males. Age-wise analysis revealed that dogs under one year of age had significantly

higher infection rates, while dogs over two years old had the lowest. Statistical analysis confirmed significant differences primarily between the youngest age group and older cohorts, with no significant differences noted among the older groups.

Infection rates also varied significantly by breed, with large breeds demonstrating the highest prevalence. The origin of dogs was another significant factor, as native breeds were more commonly infected with the enteric variant of canine coronavirus compared to imported breeds (Table 4).

Analysis confirmed that the dog samples from Mosul, Iraq, contained specific canine coronavirus (CCoV) genetic sequences. Of all submitted samples, five isolates were successfully sequenced and uploaded to GenBank, with accession numbers PV454546.1, PV454547.1, PV454548.1, PV454549.1, and PV454550.1.

A molecular analysis of the CCoV SIIa gene sequences was performed using BLAST alignment against the NCBI database to compare the local isolates with globally registered reference strains. The isolates showed varying degrees of similarity to previously published CCoV sequences. Phylogenetic analysis revealed that several local isolates shared 100% nucleotide identity with international reference strains, including GQ148772.1 (Hungary), GU146061.1 (Italy), and OQ407503.1 and OL687146.1 (China). The lowest similarity was observed with strain EU924791.1, which had 98.06% sequence identity (Table 5).

These findings confirm a close genetic relationship between local Mosul isolates and global CCoV strains. The data were further used to construct a phylogenetic tree that clarified the evolutionary position of the local isolates within the broader CCoV lineage.

Table 4: Enteric Canine Coronavirus (CCV) Infection Detected by RT-PCR

| Factors | | No. tested animals | Total positivity using q RT-PCR (%) | P value |
|---|---|---|---|---|
| Animals management | Household | 33 | 14(42.4) [a] | 0.000 |
| | Stray dogs | 17 | 13(76.4) [b] | |
| Gender | Females | 29 | 10(34.4) [a] | 0.478 |
| | Males | 21 | 17(80.9) [b] | |
| Age | < 1 year | 22 | 14(63.6) [a] | 0.000 |
| | ≥ 1 year – 2 years | 18 | 10(55.6) [b] | |
| | ≥2 years | 10 | 3(30) [c, b] | 0.003 |
| Breed | Small breed | 20 | 5(25) [a] | 0.000 |
| | Large breed | 30 | 22(73.4) [b] | |
| Source | Native | 27 | 20(74) [a] | 0.000 |
| | Imported | 23 | 7(53.8) [b] | |

Table 5: Comparability of International GenBank Strains and Local Canine Coronavirus Sequences Using NCBI BLASTn

| Local sequences Accession No. | Pathogen Identified | Name of Gene | Identic Number % | GenBank Accession Number | Country Identification |
|---|---|---|---|---|---|
| | Canine coronavirus | spike protein | 100 | OQ407503.1 | China |
| PV454546.1 | | | 100 | OL687146.1 | India |
| PV454547.1 | | | 98.72 | OL687147.1 | India |
| PV454548.1 | | | 99.75 | DQ112226.1 | Italy |
| PV454549.1 | | | 100 | GU146061.1 | Italy |
| PV454550.1 | | | 98.06 | EU924791.1 | Italy |
| | | | 100 | GQ148772.1 | Hungary |

Sequence alignment between the five local canine coronavirus isolates and reference sequences from the GenBank database revealed similarity rates ranging from 93.08% to 100% (Table 6).

CLUSTALW alignment analysis of the local SIIa gene sequences demonstrated a high degree of sequence similarity among the isolates, with alignment scores ranging from 93.08% to 100%. These findings suggest that the local strains are highly conserved.

Table 6: Alignment Score Between and Within Local Canine Coronavirus Sequences Using Multiple Sequence Alignment (CLUSTALW)

| Sequences Accession No. | Alignment score |
|---|---|
| PV454546.1<br>PV454547.1<br>PV454548.1<br>PV454549.1<br>PV454550.1 | 93.08 - 100 |

Phylogenetic analysis of the five local canine coronavirus SIIa genotypes revealed evolutionary relationships with previously identified international strains. The phylogenetic tree constructed in this study showed genetic relatedness ranging from 68% to 96%. Notably, strains from China and Italy exhibited the highest genetic similarity to the local isolates (Figure 2).

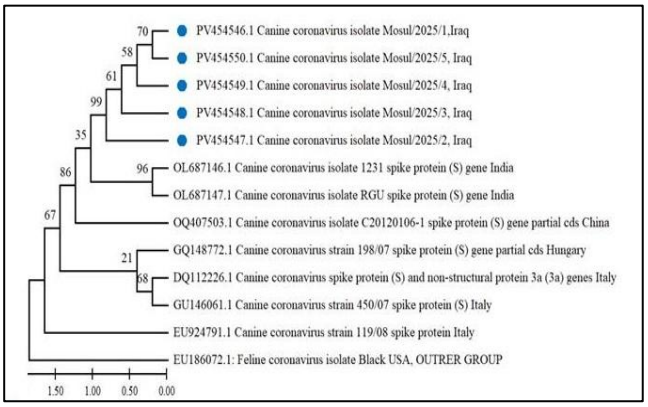


Figure 2: Phylogenetic Tree of Canine Enteric Coronavirus-IIa Isolates from Mosul, Iraq.

The phylogenetic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model in MEGA11 software, with 1,000 bootstrap replicates to assess branch reliability. The analysis utilized partial sequences of the spike (S) protein gene, with the feline coronavirus strain EU186072.1 (USA) serving as the outgroup.

**Discussion**

Enteric Canine Coronavirus is a significant concern in dogs due to the severe clinical symptoms it causes, particularly in infected animals. The infection is especially dangerous in young dogs and can sometimes result in death. Because of this heightened risk, especially in puppies, achieving an accurate and rapid diagnosis is a primary priority in controlling the spread and impact of this virus (12).

The detection and reported prevalence of canine coronavirus (CCoV) in canine populations have shown considerable variability across studies, largely due to differences in diagnostic methods, study populations, and regional factors. In the current investigation, antigen detection from rectal swabs indicated that 54% (27 out of 50) of tested dogs were positive for CCoV, highlighting a substantial burden of the virus within this cohort. This finding aligns closely with the observations of (21), who, employing the more sensitive nested polymerase chain reaction (PCR) technique, reported 54 positive samples among 58 tested. The heightened sensitivity of molecular techniques such as nested PCR is likely reflected in the elevated detection rate compared to antigen tests. In contrast, other studies have documented lower prevalence rates. For instance, (22) screened 109 dogs for both CCoV and canine parvovirus (CPV), revealing only five CCoV-positive cases in the population, compared to 37 for CPV. Notably, this study found no evidence of mixed infections, suggesting distinct epidemiological patterns for each virus. Additional data from (23) and (24) offered broader prevalence estimates, noting CCoV in 8% of sampled dogs and CPV in 50%. These results underscore how prevalence is influenced by factors such as testing strategy, population health, and regional virus circulation. Molecular surveillance by (25) revealed that CCoV was the third most prevalent enteric virus in their surveyed population, with a 20% detection rate. Their report also highlighted the occurrence of double and triple infections, further illustrating the complexity of enteric viral infections in canines. A comprehensive analysis by (26) provided a pooled prevalence of 33% for CCoV infection, with an apparent concentration among younger dogs. This meta-analysis found no significant association between infection rates and either sex or vaccination status, and identified CCoV-II as the predominant subtype circulating among Chinese dogs.

In our study,the variation in infection rates of canine enteric coronavirus (CECoV) among dog populations is influenced by multiple factors, including the diagnostic methods employed, the accuracy and rigor of viral surveillance across countries, and disparities in veterinary healthcare access. Diagnostic sensitivity plays a key role; molecular assays such as PCR often detect more cases than antigen-based tests. Moreover, differences in immune status and vaccination practices—particularly the use of core versus non-core vaccines—critically shape infection dynamics and prevalence (27).

Our findings revealed a notably higher prevalence of CECoV infection in younger dogs, especially those aged ≤3 months. This concurs with previous studies (28-29), (24) indicating that the youngest puppies are most vulnerable. Immature immune systems and delayed immunization schedules contribute substantially to this increased susceptibility, while breed diversity may modulate host immune responses. Supporting epidemiological evidence includes (30), who reported 41.86% CPV infection among Assamese puppies aged 4–6 months and 26.67% CECoV infection in those ≤3 months. (31) documented CPV prevalence rates of 59.25% in 1–3-month-old puppies and 37.5% in 3–6-month-olds. Consistent with this, our study detected CECoV infections solely in the ≤3-month age group, reflecting a critical early-life window of susceptibility. (32) observed higher infection rates among younger dogs, partially explained by increased owner vigilance regarding health and vaccination. Contrarily, some

studies report elevated susceptibility in older dogs, potentially due to waning antibody titers. Nonetheless, the prevailing consensus holds that puppies under six months, especially those younger than three months, remain at highest risk. In regions lacking CECoV vaccination programs, absent or low maternal antibodies further exacerbate vulnerability. While maternal antibodies impart some protection, subgroup analyses suggest they may be insufficient against CECoV as titers decline. Addressing this gap through optimized vaccination timing and maternal immunization strategies should be prioritized. Stray dogs demonstrated significantly higher infection rates than household pets. This disparity is likely attributable to the near absence of vaccination in stray populations and their increased exposure to infectious agents through social interaction and inadequate shelter. Conversely, household dogs typically inhabit less crowded environments, receive regular veterinary care, and are often vaccinated against multiple pathogens, promoting robust humoral and cellular immune responses that confer better protection. Our data indicate that local dog breeds have a higher CECoV prevalence than imported dogs. Although imported dogs are clinically known to be susceptible to diverse viral infections, most imported animals in our study had comprehensive vaccination histories and veterinary oversight, explaining their lower infection rates. While vaccine failure can occur due to immunosuppression, antigenic mismatch, or insufficient immune response, this was not evident in our imported group, underscoring the importance of effective vaccination. Male dogs recorded higher CECoV prevalence, consistent with **(**33), potentially reflecting behavioral tendencies such as aggressive encounters and increased social contact. Females, however, may face heightened susceptibility during physiological stressors like pregnancy and estrus. Large breed dogs exhibited greater infection rates, likely because they are often raised outdoors or on farms and used as working or guarding animals, all of which increase pathogen exposure. Small breeds, generally kept indoors as companions, benefit from reduced environmental interactions.

Infected dogs commonly showed multiple clinical signs typical of viral gastroenteritis, with watery diarrhea being most prevalent. (34) similarly reported vomiting, diarrhea, and inappetence as hallmark CECoV symptoms. (35) found higher prevalence in apparently healthy dogs compared to clinically ill ones, suggesting significant subclinical infection or varied immune responses among individuals.

Genetic analysis of CECoV isolates from Mosul revealed diversity with sequence similarity ranging from 93.08% to 100%. Several local strains exhibited 100% nucleotide identity with established international references from China (OQ407503.1, OL687146.1) (36,37), Italy (GU146061.1) (38), and Hungary (GQ148772.1) (39), while the lowest similarity was noted with the EU924791.1 (40) strain. Such genetic variability results from mutations facilitating adaptation to host immune responses, potentially driving emergence of new strains with varying virulence and outbreak potential. Environmental and geographic factors likely contribute to local strain divergence. Phylogenetic trees revealed subgroups distinct from global strains, indicating shared evolutionary origins alongside regional divergence. Continued molecular surveillance is vital for monitoring viral evolution and guiding effective vaccine strain selection (41).

## Conclusions

dogs are afflicted with CECOV, and Nineveh province has a higher prevalence with IIa subtype

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## Conflict of interest

The author declares that there are no conflicts of interest regarding the development, writing, or submission of this manuscript.

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# التوصيف الجيني للفيروسات التاجية المعوية الكلبية النمط IIa من الكلاب في مدينة الموصل

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**الخلاصة**

شملت الدراسة ٥٠ كلبًا من مناطق مختلفة في محافظة نينوى، العراق، حيث تم جمع مسحات شرجية منهم للكشف عن الفيروس التاجي الكلبي (CECoV) تنوعت الكلاب من حيث الفئات العمرية، الجنس والسلالات. تم استخلاص الحامض النووي الفيروسي (RNA) من المسحات الشرجيّة، ثم أجري الكشف الجزيئي للنمط الجيني للعترات الفيروسية باستخدام تقنية تفاعل البوليميراز المتسلسل بواسطة النسخ العكسي (RT-PCR) بعد ذلك، استُخدم ناتج التفاعل لإجراء التسلسل النيوكليوتيدي بواسطة طريقة سَنجَر، وأُجري تحليل تطوري وبناء شجرة وراثية باستخدام برنامج MEGA X لمقارنة العترات المحلية بالسلالات الفيروسية العالمية المسجلة. أظهرت النتائج أن ٢٧ من أصل ٥٠ عينة (بنسبة ٥٤%) كانت إيجابية لفيروس الكاسي الكلبي. لوحظت أعلى معدلات الإصابة في الكلاب الضالة، مع عدم وجود فرق إحصائي دال مقارنة بالكلاب المنزلية. ومن حيث الجنس، سجلت الإناث معدلات إصابة أعلى. أما من ناحية العمر، فكان معدل الإصابة الأعلى في الكلاب التي تقل أعمارها عن سنة واحدة. ووفقًا لتحليل السلالات، لوحظ تباين كبير في معدلات الإصابة بناءً على المصدر والسلالة. فقد أظهرت الكلاب من السلالات الكبيرة معدلات إصابة أعلى مقارنة بالسلالات الصغيرة، كما كان معدل الإصابة أعلى في السلالات المحلية مقارنةً بالسلالات المستوردة. تم الكشف عن وتسلسل خمس عينات بنجاح، وسُجلت في بنك الجينات.أظهرت الشجرة التطورية أن هذه السلالات المحلية من نمط S IIa تحمل علاقات تطورية مع العترات الدولية المسجلة سابقًا، حيث تراوحت درجة القرابة بين ٦٨% و٩٦..%.