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Clinical and serological study of canine parainfluenza virus-5 in dogs in Baghdad province

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Abstract
This study aimed to evaluate the vital and clinical signs of Canine parainfluenza virus-5 (CPIV-5) infections in dogs, and identify the specific IgM and IgG antibodies against the virus by the indirect methode of Enzyme Linked Immunosorbent Assay (ELISA). 150 blood samples were obtained from 100 dogs exhibiting clinical respiratory symptoms (symptomatic) and from 50 apparently healthy dogs (asymptomatic), vital and clinical signs of all dogs were documented during January 2023 to April 2024 in Baghdad city. Clinical examination showed that infected dogs had symptoms such as nasal discharge, in appetence, lethargy, coughing that is dry hacking and persists for a period of time in some cases, fever, abnormal breath noisy, pallor mucous membrane, ocular discharge, conjunctivitis and in some severe cases mouth breathing. Diarrhea, vomiting and dehydration were observed in certain dogs. A significant rise in body temperature (40.19±0.08ºC), pulse rate (123.3±1.04 beat/min) and respiratory rate (35.59±0.26 breath/min) was noticed compared with asymptomatic dogs. The results of serological study by CPIV-5 IgM ELISA test proved significant variation the positivity rates between symptomatic and asymptomatic dogs, the seropositivity rate of CPIV-5 IgM antibodies was 86/100 (86%) in symptomatic dogs, in contrast, all the asymptomatic dogs were seronegative. The seropositivity rate by using CPIV-5 IgG ELISA test showed a significant variation in the positivity rates between symptomatic and asymptomatic dogs, the higher positivity rate for CPIV-5 IgG antibodies appeared in asymptomatic dogs 31/50 (62%), while the rate lowered to 32/100 (32%) in symptomatic dogs. This study is the first attempt to identify IgM and IgG antibodies against CPIV-5, and evaluate the disease's vital and clinical features in dogs in Baghdad, Iraq.

Keywords: Baghdad, Iraq, parainfluenza, CPIV-5, Clinical, IgM, IgG, ELISA.

Introduction
Parainfluenza virus-5 (PIV-5) is a paramyxovirus that has been isolated from numerous mammalian hosts and is well-known for its capacity to produce persistent infections, particularly noted in veterinary medicine, where it is known as canine parainfluenza virus-5, the principal viral pathogen of dogs (Hankinson *et al*., 2025; Randall *et al*., 2025) [26, 39]. Animals with acute infections expel it from their respiratory tracts, making it a highly contagious disease (Buonavoglia and Martella, 2007) [11]. It is a common zoonotic virus that causes "canine infectious respiratory disorder" (CIRD), commonly referred to as "kennel cough," in dogs (Cheng, *et al*., 2023) [13]. Where single or multiple viral and bacterial pathogens are involved sequentially or synergistically to cause diseases (Maboni *et al*., 2019; Cordisco *et al*., 2022) [33, 14]. When dogs are kenneled or brought together for events like dog shows or athletic competitions, CPIV-5 epidemics are prevalent due to its high transmissibility (Ford, 2006; Mitchell *et al*., 2017) [23, 35]. Direct or indirect contact with respiratory secretions, or aerosols, and/or infected objects, like clothing, water or food containers, and bedding, are the most frequent ways that the disease is spread (Berliner, 2021) [9]. In dogs two weeks of age or older, the illness is typically limited to the upper respiratory tract (Tiwari *et al*., 2020) [43]. The virus's main clinical symptoms include fever, conjunctivitis, serous nasal discharge, and a loud or honking cough (Ellis, 2021) [17]. Lethargy, fever, in appetence, and pneumonia are some of the symptoms that may worsen in dogs co-infected with *B. bronchiseptica*, the more complex forms of the infection are typically seen in immunocompromised animals or young,

unvaccinated puppies (Tiwari *et al*., 2020) [43]. In general, several diagnostic methods were used to detect viral infections in different hosts, such as the real time quantitative polymerase chain reaction (RT-qPCR), virus neutralization (VN), hemagglutination inhibition (HI), immunocgromatograghy (ICG), ELISA, and gel precipitation (Tizard, 2009; Jeoung *et al*., 2009; Al-Saadi, 2022; Dall'Ara *et al*., 2023; Amanee, 2023) [44, 30, 5, 15, 6]. A popular technique for detection of viral antigen or antiviral antibodies, is the ELISA test, this test has the advantage that both positive and negative controls can be incorporated with the test serum in one well. In addition to the serum, whole blood, plasma, or saliva may be employed as a source of antigen or antibody (Tizard, 2009) [44]. Furthermore, the PIV-5 strain's ability to infect a variety of mammalian cell types suggests that it can spread to other species, offering a startling clue about possible zoonosis. Therefore, the pathophysiology, epidemiology, and zoonotic potential of the virus can be examined using ELISA (Ibrahim, 2022; Moas and Zenad, 2020; Hamzah and Mosa, 2020) [28, 36, 24]. Numerous studies had been conducted on canine in Iraq (Amanee *et al*., 2024; Hussein & Al-Graibawi, 2024; Jbr & Jumaa, 2024; Ali & Yassein, 2022; Mansour & Hasso, 2021; Badawi, & Yousif, 2020; AL-Mutar, 2020; Tamimi & Wali, 2019; AbdulKareem *et al*., 2020; and Hamzah, *et al*., 2019) [7, 27, 29, 2, 34, 8, 4, 42, 1, 25]. It is worth mentioning that there are no previous studies on the CPIV-5 in Iraq. So, this study aimed to describe clinical features and detect of IgM and IgG antibodies to CPIV-5 in dogs.

Material and methods

Sample collection

One hundred and fifty (150) blood samples were taken from a population of 150 Dogs (100 dogs with respiratory signs (symptomatic) and 50 clinically healthy dogs (asymptomatic). The samples were gotten from the different area of Baghdad city which includes (Baghdad veterinary hospital, private veterinary clinics) from January 2023 to April 2024. Clinical inspection done, the vital and clinical signs were documented. Additionally, key data such as age, sex and breed for each dog was obtained from the dog's holder.

Samples preparing

Blood was collected aseptically via puncture of the cephalic vein (Yagi and Holowaychuk, 2016) [46]. Gel and clot activator vacuum tubes were used for serum separation that was separated by centrifugation at 5000 rpm for 3 minutes. The sera were kept at-20°C up to use.

Serum Analysis

Samples were examined for IgM and IgG using commercial ELISA Kits (Ideal medical Shanghai) according to the manufacturer's instructions. Within ten minutes of adding the stop solution, the optical density (OD value) of each well was measured right away using a microplate reader set to 450 nm.

Ethical Management of the Study

The present study was approved in agreement with guidance issued by College of veterinary medicine, University of Baghdad. No banned biological materials or genetically modified organisms were included in the report (No. P.G/199 at 25/1/2023).

Statistical Analysis

The data were evaluated by using the chi-square analysis and fisher's exact calculation methods, and P values below 0.05 were accepted as statistically significant (Bluman, 2017) [10].

Results and Discussion

Clinical study

This is the first research focus on the vital and clinical manifestations and prevalence of CPIV-5 IgM and IgG antibodies in dogs in Iraq using ELISA test. In current study, the clinical examination revealed that symptomatic dogs suffered from several clinical signs including, nasal discharge, in appetence, lethargy, coughing which is dry hacking persists for a period of time in some cases, fever, conjunctivitis with ocular secretion, abnormal breath noisy, pallor mucous membrane where their percentages (80%, 78%, 78%,77%, 75%, 58%, 37% and 35%) respectively. In contrast, diarrhea, vomiting, mouth breathing and dehydration were (23%, 17%, 11% and 7%) respectively, table (1)

Table 1: The clinical signs occurrence in symptomatic dogs

Clinical signs	No	Percentage%
Nasal discharge	80	80
In appetence	78	78
Lethargy	78	78
Coughing	77	77
Fever	75	75
Conjunctivitis with ocular secretion	58	58
Abnormal breath noisy	37	37
Pallor mucous membrane	35	35
Diarrhea	23	23
Vomiting	17	17
Mouth breathing	11	11
Dehydration	7	7
Total of sick dogs	100	

They were in contract with (Tiwari, *et al*., 2020; Ellis, 2021; Cordisco, *et al*., 2022) [43, 17, 14] whom showed the clinical signs of CPIV-5 infection including: Serous or purulent nasal discharge, conjunctivitis, retching, tonsillitis, pharyngitis, fever, lacrimation, depression, dyspnea, diarrhea, and a paroxysmal, severe cough lasting two to six days. In addition the virus invades the respiratory epithelium rises the susceptibility to secondary infection, because of the loss of cilia and death of ciliated epithelium, infection causes more severe respiratory disease when it is complicated by co-infection with other viruses and bacteria. This disruption of the mucociliary escalator's normal housekeeping function may also make secondary infection more likely (Priestnall and Sykes, 2021) [38]. A significant rise in body temperature (40.19±0.08°C), pulse rate (123.3±1.04 beat/min) and respiratory rate (35.59±0.26 breath/min) was noticed compared with asymptomatic dogs (Table 2, Figure 1).

Table 2: Vital value in symptomatic and asymptomatic dogs (range and mean ± standard error)

Parameters	Symptomatic dogs	Asymptomatic dogs
Body temperature C°	*38.4-41.5(40.19±0.08)	37.7-39.5 (38.48±0.05)
Pulse rate\ minute	*95-132 (123.3±1.04)	65-122 (93.26±2.62)
Respiratory rate\ minute	*30-40 (35.5±0.2)	15-31 (23.1±0.7)

*p<0.0001 significant difference

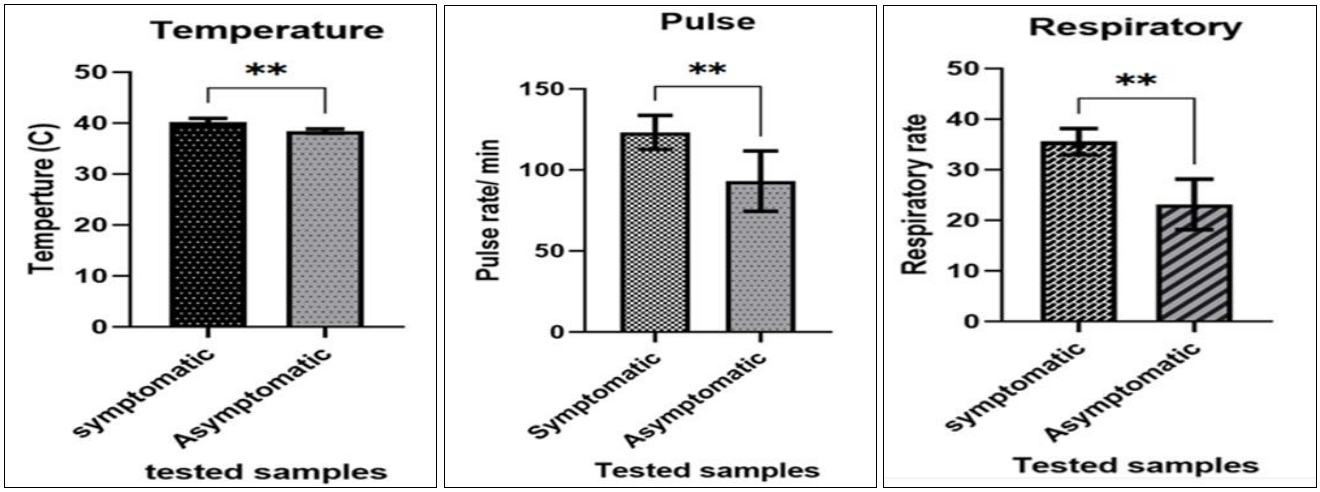

Fig 1: Shows temperature, pulse rate and respiratory rate in symptomatic and asymptomatic dog.

The parameters measured (body temperature, pulse and respiratory rate) are key indicators of physiological stress and disease progression, the hyperthermia reflects an acute phase immune response to infections or inflammation, commonly observed in CRID such as CPIV-5 (Sykes, 2014) [41]. As well, the pulse rate in symptomatic dogs was considerably higher than in asymptomatic dogs that could be as a result of systemic fever, stress, or dehydration (Feldman *et al*., 2015) [22]. Respiratory rate also increased in symptomatic dogs may be linked to respiratory tract inflammation, obstruction of airway, or impaired gas exchange, which are characteristic signs in ill dogs (Ford, 2006) [23].

Results of CPIV-5 IgM ELISA test

The serological examinations by CPIV-5 IgM ELISA test in the present study revealed significant variation in the positivity rates between symptomatic and asymptomatic dogs, 86 out of 100 symptomatic dogs (86%) were positive for CPIV-5 IgM analysis, whereas all asymptomatic dogs were seronegative, figure (2).

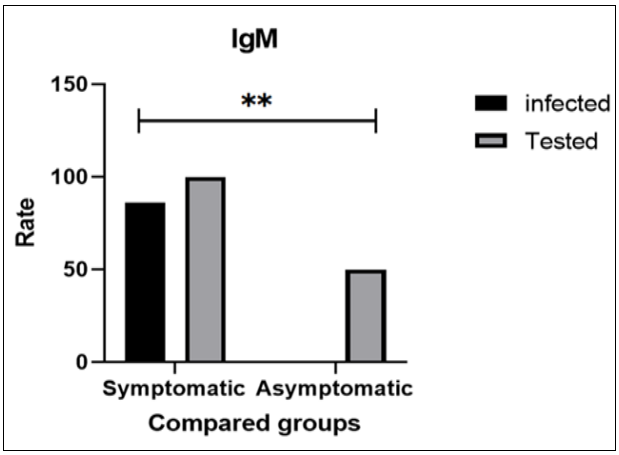

Fig 2: The percentage of CPIV-5 IgM in symptomatic and asymptomatic dogs

The results was statically significant (p<0.0001) indicating a strong association between clinical signs and recent infection, moreover, the current investigation confirmed the presence of IgM in symptomatic dogs due to the immunity response to CPIV-5, which includes both systemic IgM antibody response and local mucosal production of IgA antibodies (Ellis and Krakowa, 2012) [18]. That could be as a result of an active viral infection because it has been most commonly shown to be present in canine respiratory diseases (Yang *et al*., 2024) [47]. The higher incidence of CPIV-5 IgM antibodies among naturally infected dogs versus apparently healthy dogs emphasizes that the virus is endemic in the region due to the fact that it is extremely contagious and effectively spread by oronasal contact with aerosolized respiratory secretions from infected dogs (Sykes, 2014) [41]. In contrast, none of the asymptomatic dogs tested positive, suggesting that no exposure to the virus or absence of acute infection stage and lack of active viral replication, or mild infections unless intensified by stressful condition or co-infections with other organisms (Buonavoglia and Martella, 2007) [11]. Mitchell *et al*. (2017) [35] reported that in addition to infectious agents, high pathogen concentrations, exposure frequency, physiological stress, and host susceptibility variations, including immunological status has been a significant correlation with the frequency of CPIV-5 and the infected dogs are more likely to have a history of recent stays in a kennel, refuge, boarding house, or pet store despite immunization, though this can also happen to dogs living alone. The results of this investigation were higher than a study by Englund *et al*. (2003) [20] who found the presence of antibodies against CPIV-5 in Sweden by using (HI) test when sera from 302 pet dogs were examined for this investigation, the virus's seropositivity was found to be 28%. Erles *et al*. (2004) [21] found the seropositivity of CPIV-5 by the evaluation of an ELISA assay was (57.9%) in 22 out of 38 dogs developed respiratory disease. Ueland (1990) [45] reported that canine infectious tracheobronchitis outbreak that appeared to be more contagious than typical occurred throughout Scandinavia in the autumn of 1988. Serum samples were taken from 52 dogs that had respiratory indications; the findings showed that the titer of antibodies against CPIV-5 increased significantly in 79% of the cases, indicating that the disease outbreak was significantly influenced by the virus. The IgM in symptomatic dogs has been previously documented in studies of other canine viral diseases, where the IgM is only detectable during the acute phase of Canine parvovirus infection (Decaro and Buonavoglia, 2012) [16].

Results of CPIV-5 IgG ELISA test

The present study revealed that the higher positivity rate for CPIV-5 IgG antibodies appeared in asymptomatic dogs 31/50 (62%), while the rate lowered to 32/100 (32%) in symptomatic dogs, there was a significant variation in the positivity rates between symptomatic and asymptomatic dogs

as shown in Figure 3.

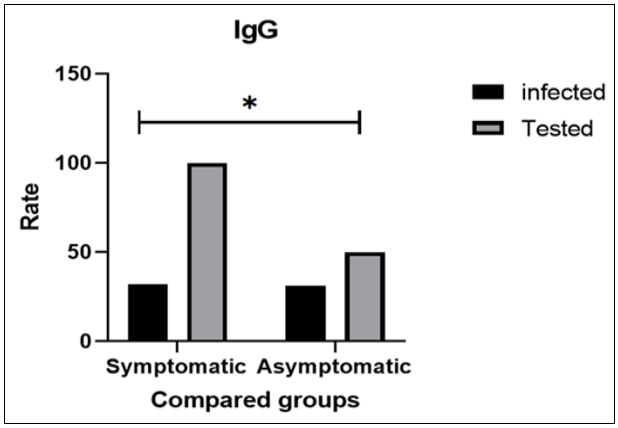

Fig 3: The percentage of CPIV-5 IgG in symptomatic and asymptomatic dogs

The prevalence of CPIV-5 IgG recorded in symptomatic and asymptomatic dogs that could have a major impact on the frequency of natural exposure to the pathogen that may have been circulating in the area all along at the population level or as a result of vaccination (Renshaw *et al*., 2010) [40]. The lower IgG rate in symptomatic dogs might reflect a more recent infection, during which IgG levels have not yet peaked, or an inadequate immune response, conversely, it was shown to be considerably higher in dogs without respiratory distress and a significant difference was seen in the total incidence of the virus suggest that asymptomatic dogs may have been previously infected by CPIV-5 or subclinical infection that developed a strong enough immune response to prevent the appearance of clinical signs (Mitchell *et al*., 2017) [35]. However, there aren't many data that address this issue at the dog's population level, in an earlier seroepidemiologic investigation Mouzin *et al*. (2004) [37] reported that vaccinated dogs with a "high risk" lifestyle (greater chances for communal interaction) were shown to have a higher chance of maintaining higher titers of CPIV-5 neutralizing antibodies. Alternatively, unknown geographic factors, including climate, may be the cause of observed variations in pathogen prevalence or transmission (Campos and Godson, 2003; Aljabory and Mosa, 2021) [12, 3]. Ellis *et al*. (2011) [19] who evaluated seroepidemiologic features of CPIV-5 in dog populations in western Canada by Indirect ELISA assay demonstrated that, out of the 125 dogs analyzed, 14 had incoming values of > 12.9 in the test utilizing CPIV-5 antigen, 101 had incoming ELISA units of < 12.9 (low or no antibodies), 41 dogs with low antibodies and 2 dogs with moderate to high antibodies to the virus had respiratory disease. There was no discernible correlation between antibodies and the avoidance of clinical symptoms of respiratory infections. The seropositivity rate was 98.3%, according to a Korean study by Yang *et al*. (2024) [47] that looked for virus-neutralizing antibodies against CPIV-5 in 400 canine blood samples collected between 2019 and 2022. Levy *et al*. (2008) [32] found that the antibodies had 100% seropositivity against CPIV-5 in 95 blood samples from the Galapagos. In another investigation, Kimber *et al*. (2000) found that blood samples taken from 64 seemingly healthy wild North American river otters from New York State had a seronegative result against CPIV-5.

Conclusions

Canine Parainfluenza virus-5 infection was widely distributed as etiological agent of canine respiratory infection in dogs in Baghdad city, the infection was manifested with mild to severe respiratory illness with ocular symptoms, so, application of control/prevention measures, including vaccination and environmental management, to diminish the circulation of this virus in canine population and improve dog health.

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Animal Rights Statement: Not applicable.

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