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Clinical Examination, Haematological Changes of Canine Parvovirus with Laboratory Detection by Rapid Antigenic Test

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Abstract

Background: Cases of canine parvovirus have increased in the last few years in Iraq.

Aims: to identify clinical, haematological changes in dogs infected with canine parvovirus

Material and Methods: A total of 50 dogs of various ages, sexes, and breeds were clinically investigated from December- February2021. Sterile swabs were used for collection of fecal samples. CPV using a commercially available quick CPV antigen detection test kit.

Results: Only 40 dogs were infected with CPV, which is clinically exhibited by enteric form lethargy, weight loss, lack of appetite, diarrhea, and develops to cause blood-tinged or bloody diarrhea, foul-smelling vomiting, and intractable fluidy diarrhea. Hematological analysis of the samples with neutropenia, lymphopenia, and monocytopenia revealed statistically significant declines (P 0.05) in the RBCs, Hb, PCV, MCV, MCH, MCHC, and WBCC. Anomalies in erythrocyte morphology included leptocytes, echinocytes, schistocytes, hypochromia, anisocytosis, and poikilocytosis. Infection with CPV more commonly among males (81.5%) than female (78.3%). CPV infection more commonly in younger ages a dog. There is a correlation between infection rates and breeds of dogs, with German shepherds and Terriers having higher infection rates (88.2% and 85.0%, respectively) than other breeds (57.1%).

Conclusions: CPV infect younger age, male dogs with significant hematological changes

Keywords: Canine parvovirus, hematology, Immunochromatography

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Introduction

The feline parvovirus subgroup includes the canine parvovirus (CPV). It is thought to be endemic to almost all populations of domesticated and wild canines. It is closely linked to feline panleukopenia virus and mink enteritis virus. Myocarditis and enteritis are two diseases that are brought on by CPV. The maternal antibodies defend against the myocardial form, making it uncommon. However, the intestinal type is common and can be fatal to young puppies and old dogs [1,2]

Severe, frequently bloody diarrhea, vomiting, leukopenia, and dehydration are all symptoms of CPV enteritis. The majority of diseases are spread through contact with contaminated feces and are transmitted fecal-orally. High levels of contagiousness and environmental stability characterize CPV. Quick CPV diagnosis enables isolation and effective treatment of infected canines [3]

The most common viral cause of enteritis and mortality in puppies is canine parvovirus (CPV). The distinctive characteristics of CPV make it an internationally reemerging disease of dogs. According to Kumar (2011) [4], parvoviruses have a 5,000 base single-stranded DNA genome with a hairpin shape.

When puppies are between 6 and 8 weeks old and beginning to wean, the CPV susceptibility window occurs. The majority of puppies that pass away from CPV do so at eight weeks of age. The genetic make-up (canine major histocompatibility antigens) of the puppies also affects variances in the destruction of antibodies and induction of active immunity following vaccination. Diagnostic assays that could swiftly assess the antibodies against various CPV subtypes in the kennel environment, genotype the virus, and determine the quantity of CPV in a sample would be clinically helpful [4, 5, 6,7].

There are a few methods that have been utilized for the quick identification of CPV in fecal samples and CPV antibodies, including the immunochromatography assay, latex agglutination test, and coagglutination test. Therefore, the authors of the current study are convinced that these

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quick, affordable, and safe tests will promote early CPV detection and assist in managing epidemics in Diyala Province.

Materials and Methods

Study Area: The Clinical Pathology Laboratory, Internal Medicine and Preventive Department, College of Veterinary Medicine, and Diyala University all participated in the study. The majority of the dogs who are brought to the private veterinary clinic for care are residents of Baqubah, the provincial capital of Diyala.

Clinical examination: Dogs are examined clinically for general body condition, signs of diarrhea and anorexia with or without vomiting, auscultation of respiratory and heart rate, mucus membrane and body temperature.

Faecal samples collection: With the aid of sterile swabs, feces were collected from the dog's rectum and evaluated using a commercially available quick CPV Ag test kit based on the immunochromatography (IC) assay technique. The manufacturer's recommended protocol was followed during the test. The appropriate steps were made to reduce pain or discomfort for the animals while adhering to all applicable international, national, and institutional standards for the care and management of animals [8,9].

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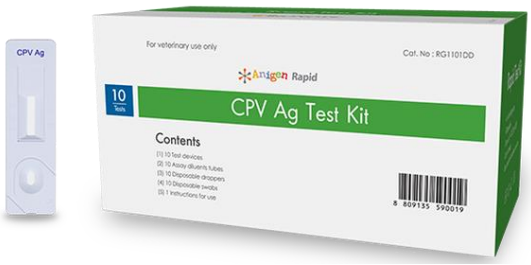

Procedure of the Test according to Bionote/Anigen Rapid Test Kits

Blood Parameters: / Canine Parvovirus Ag (CPV Ag)The blood sample was collected from Cephalic vein to estimation of blood parameter [(RBC) , (Hb), (PCV), WBC, (DWBC) and Indirect platelets count]. The blood was collected into vials containing sodium ethylenediamine tetracetic acid (Na2 EDTA) sufficient for 2.5 mL of blood to prevent coagulation. The tubes were gently rotated to ensure proper mixing of the blood with the anticoagulant without damaging the integrity of the cells and were transported to the laboratory [8,9].

Data of Collection and Analysis : For each dog tested, data was collected regarding age, sex, breed, clinical examination also nature of diarrhoea (haemorrhagic or nonhaemorrhagic) and CPV vaccination history and recorded accordingly. The data was analyzed using SPSS version 23 and was subjected to Chi-square test at $P<0.05$.

Results

Clinical signs: The total numbers of dogs examined clinically were 50 during (December, January and February 2021) in different ages, sex and breeds. Only 40 dogs were infected by CPV represented clinically by enteric form lethargy, weight loss, diarrhea which represented with progresses the infection by blood tinged or bloody diarrhea, foul smelling, intractable fluidy vomiting as in (Fig. A,B,C,D,E,F). On the other hand, Cardiac form represented by tachycardia, pale of mucus membrane,

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irregular heartbeats and dyspnea

Figure 1.A,B,C,D, E,F: A,B,C,D: Doberman dog and (F) German shepherd dog Passing from depression, loss of appetite, weight loss, bloody diarrhea (E) infected by

Hematological Parameter: The hematological analysis showed statistically a significant decreases ($P<0.05$) ; in the mean of red blood cells count (RBCC), white blood cells count (WBCC), hemoglobin concentration (Hb) ,packed cell volume (PCV). Erythrocytes indices Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MHCH) show decrease in values below normal range with macrocytic hypochromic anemia,and microcytic hypochromic anemia. While leukogram show leukopenia with neutropenia, lymphopenia and monocytopenia as in (Table.1). On the other hand, direct microscopic examination of blood film showed abnormalities in erythrocytes morphology represented anisocytosis in which spherocytes and poikilocytosis in which leptocytes, echinocytes, schistocytes, hypochromasia as in (Fig.2)

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Table.1 Hematological parameter of infected dogs

Hematological parameter	Healthy dogs* Mean ± SE	Infected dogs Mean ± SE
RBC (× 106/µL)	6.75±0.10	**4.200±1.101** ↓
Hb (g/dl)	16.28±0.22	**7.1 ± 0.4**
PCV (%)	47.24±0.86	**22.25 ± 2.9** ↓
MCV (fl)	70±0.38	**58.79 ± 6.27** ↓
MCH (pg)	24.1±0.18	**17.77 ± 1.4** ↓
MCHC (g/dl)	34.4±0.24	**27.48 ± 1.8** ↓
PLT (× 10 3/µL)	299±12.3	
DWBC (Absolute)		
WBC (103/µL)	9.29±0.39	**7.0± 3.4** ↓
Lymphocytes (103/µL)	2.48±0.17	**1.120± 3.5** ↓
Segmented Neutrophils (103/µL)	6.23±0.35	**2.415 ± 23.3** ↓
Band Neutrophils (103/µL)	0.0	0.07± 0.1 ↑
Monocyte (103/µL)	0.22±0.05	2.870± 0.7 ↑
Eosinophils (103/µL)	0.38±0.07	**0.455± 1.18** ↓

* Normal Mean of hematological parameter of healthy dogs in Iraq [10].

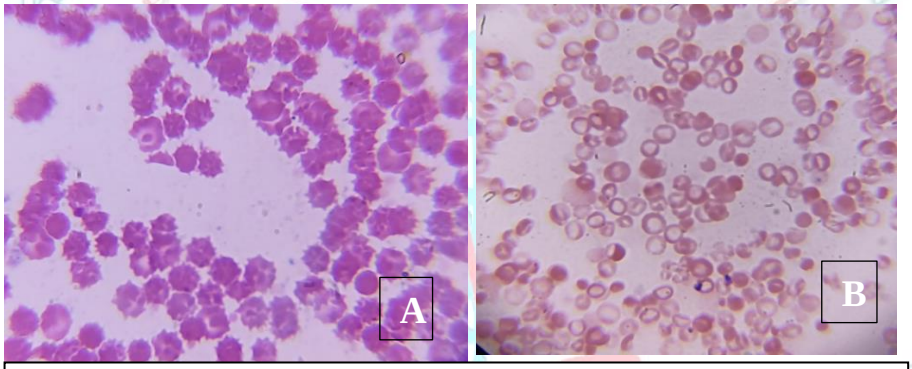

Figure 2.A,B: Abnormalities in erythrocytes morphology represented anisocytosis in which spherocytes and poikilocytosis in which leptocytes, echinocytes, schistocytes,(A) hypochromasia (B) infected by CPV infection

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Canine parvovirus antigen rapid test

Of 10 dogs passing bloody diarrhea, a fecal examintion was performed by CPV Ag -rapid test (Fig. 3) to detection of CPV infection (8/10) with infection rate 80% as (Fig. 4)

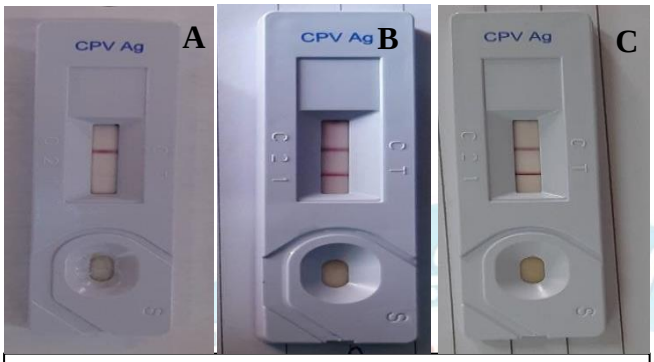

Figure 3. A, B, C: A. Negative of CPV Ag -rapid test, B, C. Positive of CPV Ag -rapid test (2 line)

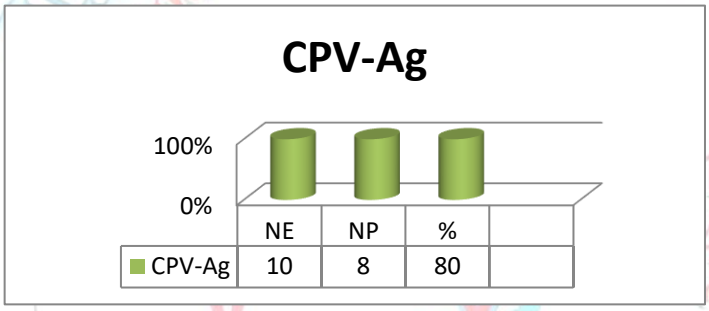

Figure 4. CPV infection (8/10) with infection rate 80%

Relation of Infection rate of CPV infection with sex of dogs.

Relation of percentage of CPV infection with sex of dogs show high infection in male 22/27 (81.5%), 17/5 than female with no significant at P<0.05 (Table. 2).

Table 2: Relation of percentage of CPV infection in dogs with sex.

Sex	Number	Positive to CPV	Infection rate %
Male	27	22	81.5%
Female	23	18	78.3%
Total	50	40	80%
X2= 0.081 *p*-value= 0.775 NSD. at P<0.05			

* X2= Chi-square , X2== Probability value , 0.775= Significant difference

Relation of Infection rate of CPV infection with age groups of dogs.

The result of relation of percentage of CPV infection with age groups of dogs show high infection rate in small age group (1-2 and 3-5 Months) 20/22 (90.9%), 18/20 (90%). While low infection rate in more than 5 months age of dogs 2/8 (25.0%) respectively (Table.3).

Table- 3: Relation of infection rate of CPV infection in dogs with age groups.

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Age	N.E	Positive to CPV	Infection rate %
1-2 months	22	20	90.9%
3-5 months	20	18	90.0 %
More than 5 months	8	2	25.0%
Total	50	40	80.0%
X2= 18.011, *p*-value= 0.00012 SD. at P<0.05			

4.6. Relation Infection rate of CPV infection with breed of dogs.

According to (Table 4) the result showed a relation of infection rate with breed of dogs which represented by high infection rate in German shepherd 88.2 % and Terrier 85.0% than other breed 57.1%.

Table- 4: Relation of infection rate of CPV infection with breed of dogs.

Breed of dogs	N.E	Positive to CPV	Infection rate %
Terrier	20	17	85.0%
German shepherd	17	15	88.2 %
Husky	6	4	66.7%
Doberman	7	4	57.1%
Total	50	40	80.0%
X2= 3.985, *p*-value= 0.263 NSD. at P<0.05			

Vaccination effect on percentage of CPV infection.

According to (Table- 4.4) the infection rate in unvaccinated dogs was 82.6% while the infection rate in dogs not receiving parvovirus vaccine were 50% with no significant at P<0.05 as in (Table 4.4)

Table 4.4. Effect of vaccination on infection rate of CPV in dogs

Variable	N.E	Positive to CPV	Infection rate %
Unvaccinated	46	38	82.6%
Vaccinated	4	2	50.0 %
Total	50	40	80.0%
X2= 2.446, *p*-value= 0.117 NSD. at P<0.05			

Discussion

Infectious enteritis in dogs is currently one of the most common diseases caused by CPV-2 infection worldwide. This illness poses a persistent threat to the survival and breeding of dogs because to its high morbidity and fatality rates, particularly in puppies [11]. According to this study, the CPV antigen has been found in the dog feces of Diyala Province for

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the first time. In order to ascertain the clinical symptoms, haematological alterations, and de tection of canine parvovirus infection via anigen Rapid CPV-Ag Test Kit in various Diyala Region locations, as well as the correlation between the infection rate of CPV and the gender, age, and breed of dogs, a study was performed.

Clinically, the primary signs of instances consist of bloodydiarrhea, either with or without vomiting, and de hydration. Similar observations have been made by (Odueko, 2019), who reported that the puppy had a temperature of 38.6°C, a pulse rate of 96 beats per minute, and a respiratory rate of 28 beats per minute, and had no prior history of receiving canine parvovirus enteritis or canine distemper vaccines. When clinical and clinical-pathological symptoms as fever, vomiting, diarrhea, and leukopenia first appeared in acutely ill animals, they already showed CPV-IgG antibodies. This is regarded as a trustworthy sign of CPV-2 disease in unvaccinated pups [11, 12, 13].

The CPV infection described in this work has previously been observed in canines all over the world [12, 13, 14], with a clear regional variation in prevalence and density. Similar findings were reported by Mosallanejad (2008) [15] . Particularly in Ghana, where the majority of clinicians rely on the appearance of profuse, foul-smelling, watery, bloody diarrhea as the primary clinical symptom to infer CPV, this particular observation is crucial to take note of [9,10].

A quick antigen test kit is one of numerous techniques for identifying virus antigen in dog feces. As a result, the particular parvovirus antibodies can accurately detect antigen in dog feces [16,17]. Canine parvovirus antigen rapid test results agreed with other investigators [9, 18]. The same outcomes were observed by Mosallanejad (2008) [15], when using the same test kit, Oh (2006) [19], Al-Bayati (2010) [10] and Kantere (2015) [6], utilized a kit to detect antibodies rather than antigen. All of these studies demonstrated good sensitivity and specificity to detect the subtypes in Iran. These tests demonstrated a high sensitivity and specificity of the Anigen Rapid - Canine Parvovirus
Antigen Test Kit, Blot Test for the qualitative detection.

The puppy was also diagnosed with Canine Parvoviral Enteritis using a commercially available quick test kit and a sample of puppy feces. The dog underwent symptomatic treatment for 10 days before being discharged on day 12 and returning for immunization on day 16 [14].

The results of the haematological alterations are shown in Table 1; these changes are associated with bloody diarrhoea and demonstrate a decrease in RBC, MCV, HB, MCH, MCHC, and leukogram value. Leukopenia brought on by viral infection of the bone marrow's rapidly dividing white blood cell progenitors. Damage to lymphocytes during the early stages of infection leads to lymphopenia. Damage to bone marrow

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precursor cells causes neutropenia. Due to DIC and decreased platelet generation in the bone marrow, platelet counts may decline in severe illness [14].

Regarding sex, male canines were more prone to infection than females, which was consistent with [20,21], observation that males were more prone than females and [22] claim that females were more prone. Also disagreed with [6,7,20], who demonstrated that it had no bearing on the frequency of canine CPV infection.

In our investigation, the majority of cases were found in puppies between the ages of 1-6 months. Puppies older than two months old who develop enteritis are likely to be most commonly affected by CPV2, which has a high fatality rate. Additionally in line with other studies, it was discovered that the infection rate was substantially higher in the young age groups (1-2 months) and (2-4 months) than in those older than 5 months old at P 0.05. This result is consistent with studies conducted in India [9,15], which revealed a greater incidence in dogs of a young age group who were more susceptible to infection. This can be explained by maternal antibodies interfering with vaccinations, inadequate vaccination practices, a lack of maternal immunity, and ineffective puppy immune systems [23].

Quinn, (2002) [24] noted that the disease was at the greatest risk between weaning and six months of age and made similar observations. The discovery, also supported by [26,27] that viral replication had been dependent on the mitotic activity of myocardial and intestinal cells at this stage of development could be held responsible for the high incidence of disease when weaning occurs as a result of a lack of maternal immunity and poor immune competency for the acquired immune response at this age.

According to (Table 4.3), the results demonstrate a relationship between infection rates and dog breeds, with cross breeds having the highest infection rates. Terrier and German shepherd dogs were discovered to be the most susceptible breeds to CPV infection in comparison to other breeds displayed during the study period. This finding is consistent with earlier reports where [9,11,14] reported low infection rates in the German Shepherd, Shi Tzu, English mastiff, and Bordeaux, which were excluded from breed-wise analysis because some of these breeds were displayed during the study period.

Unvaccinated dogs were more vulnerable to infection than dogs who had received CPV vaccinations, as shown in Table (4.4), which summarizes the infection rate of CPV infection in dogs. This is consistent with [25,26] research, which found a prevalence of 64% in unvaccinated dogs and 50% in vaccinated canines. In this investigation, there was no relationship between the dogs' vaccination status and their vulnerability to contracting CPV.

This was in line with [25,27] research, which concluded that vaccination status was not a risk factor for CPV infection, but it was at odds

with more recent research by [28,29] which suggested that vaccination status was dependent on CPV susceptibility and thus supported the idea that dogs who had received vaccinations were protected from the disease. It's possible that irregular vaccination schedules or the use of poorly maintained vaccines contributed to the high number of positive cases in this study's immunized dogs [25,26,30]

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