Detection of canine parvovirus in Iraq by using rapid antigen test kit and Haemagglutination –inhibition test

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Abstract

This study considered to be the first in Iraq conducted for detection of canine parvovirus from dogs. The virus was detected in pups brought to private Vet. Clinics in Baghdad. Pups suffered from bloody diarrhea, vomiting, dehydration and increased body temperature. Canine parvovirus antigen detected by using rapid antigen test kit and Haemagglutination – inhibition tests (HI). The result of rapid test showed the ratio of positive samples was 66.6%.

The fecal samples were checked for Haemagglutination potential using different types of erythrocyte which include rabbit, chicken, horse and dogs and showed high potential HA activity if this test performed early at the time of sample collection. The values ranging from 16- 512 Haemagglutination units. Serological characterization using of Haemagglutination–Inhibition test by using anticanine parvovirus antibodies which were prepared against standard attenuated vaccine in rabbit which gave titer of 64 Haemagglutination–inhibition . Other fecal samples checked for HI and gave high titer.

تشخيص فيروس بارفو الكلاب باستخدام العدة التشخيصية السريعة واثباط التلازن الدموي

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الخلاصة

تعتبر هذه الدراسة الأولى في القطر تهدف لتشخيص وعزل فايروس البارفو من الكلاب. تم تشخيص الفيروس بصورة رئيسية من الكلاب الوافدة إلى العيادات البيطرية في بغداد والتي تعاني من الإسهال الدموى والتقيؤ والانكاز مع زيادة في درجة حرارة الجسم. حيث تم جمع عينات البراز من هذه الحيوانات وتثبيتها باستخدام العدة التشخيصية للفحص السريع لبيان نسبة الإصابة للعينات التي جمعت حيث لوحظ إن نسبة الإصابة هي 66.6 %. ثم اجري فحص قابلية الفيروس على إحداث التلازن الدموي بالنسبة للعينات مع أنواع مختلفة من كريات الدم الحمر حيث اظهر إمكانية عاليه للفحص عند إجرائه مبكراً وبدون تأخير وتراوحت القيم بين 16 – 512 وحده تلازنية. و تم إجراء التوصيف المصلي للعينات باستخدام فحص اثباط التلازن حيث كان المعيار 64 اثباط تلازن واستعمل مصل مضاد فائق المناعة محضر في الأرانب ضد العترة اللقاحية.

Introduction

Canine parvovirus 2 was the most important viral cause of enteritis in puppies over the age of two months (1). The virus, which infect rapidly dividing cells in the intestinal epithelium leading to crypt necrosis and dilation followed by villous atrophy that is diagnostic for CPV2 infection (2). Canine parvovirus 2 was emerged in 1978

worldwide (3) and termed as CPV type 2 to distinguish it from CPV type 1 (minute virus of canine). The disease is highly contagious disease of canine which causes vomiting and diarrhea. Feces typically appeared yellow to gray contain blood or mucous and fever (40-41) ºC (4,5), and may leads to rapid dehydration (6). The disease is more severe in young puppies from weaning to six month of age (7). More than 80% of adult dogs show no symptoms of he disease (8).

Mortality rate of these infected pus is between 16-48% but may reach to 91% in untreated cases (9). Leukopenia may be present with death occurring as little as two days after onset of disease. Myocarditis may develop due to in utero exposure or under eight weeks of age (8,10). The aim of this study to identify presence of parvovirus in pups of Iraq.

Materials and Methods

- **Clinical samples:** The study included ninety (90) fecal samples were collected from clinically suspected pups, which were brought at Adan Square Vet. Clinics and Animal House Vet. Clinic in Baghdad. These cases were recorded with clinical signs of anorexia, emaciation, vomiting, foul-smelled diarrhea (mostly mixed with blood), fever, depression and lethargy.
- **Haemagglutination test (HA):** Many types of erythrocyte were used in this study to conduct haemagglutination test. Rabbit RBCs which collected from the heart of rabbit in anticoagulant tube and washed three times and used at concentration of 1%, Canine RBCs, which is collected from radius artery and the heart of dogs. Chicken RBCs were also used by same procedure but and collected from wing . Horse RBCs collected from jugular vein, all these washed RBCs, kept in refrigerator until used.
- **Rapid antigen test kit:** Rapid Monoclonal Antibody Test. This was performed and described in leaflet of kit (ANIGEN Company, KOREA) to detect CPV2 antigen in canine feces.
- **Haemagglutination – inhibition test (HI):** This test which was done with presence of Antiserum prepared in rabbit against vaccine strain (Forte Dodge, USA).

Results

Most of the cases of parvovirus infection used in this study were at age 1-6 months. There are few cases more than six months to 1 year. The clinical signs of the cases started with vomiting, diarrhea, foul smell feces, dehydration, sunken eyes, dullness and lethargy, unclotted blood was mostly observed in feces. Most untreated cases died within 3 days of illness. The disease observed in dog breeds, Rottweiler, Germanshephered and Doberman. The results are shown in table (1).

Result of rapid test, this test is chromatographic immune assay for qualitative detection of CPV antigen in canine feces. The specially selected parvovirus antibodies were used in test band as both capture and detector materials. Results of our study which performed it early at the time of samples were taken was 66.6 % (60 sample from 90) Table (2). Most of these cases which die with in 3 days after onset of clinical signs.

This study showed that there are CPV2 in Iraq because of this kit could detect this viral antigen. This kit have been found to be highly specific (98.8%) and sensitive (100%). Advisably from manufacturer do not depend on this test alone if use it in scientific research but must support by other test to increase accuracy. Fig. (1), shows of positive and negative result s of rapid test .

The positive samples also detect of HA potential to increase confirmative information about virus. Similarly, HA test was performed to detect of CPV from

infected feces by using avian and mammalian RBCs. The titer of the samples ranging from 16- 512 HA unit distributed as in Table (3), however horse RBCs showed negative for HA potential Fig. (3).

HI test is serological test using hyper immune sera raised in rabbit against vaccinal strain. The samples gave HA titer 512 and at HI, the results with these samples 64 HI units. Nevertheless, when using other two samples that carry HA at 64 and give high titer in HI 1024.

Canine parvovirus in these samples agglutinate the erythrocyte of rabbit, dog and chicken. However, it observed the virus capable to agglutinate chicken erythrocyte and these RBCs showed results of HA within 25-30 minute Fig. (2).

Table (1) Distribution of risk factors of CPV2

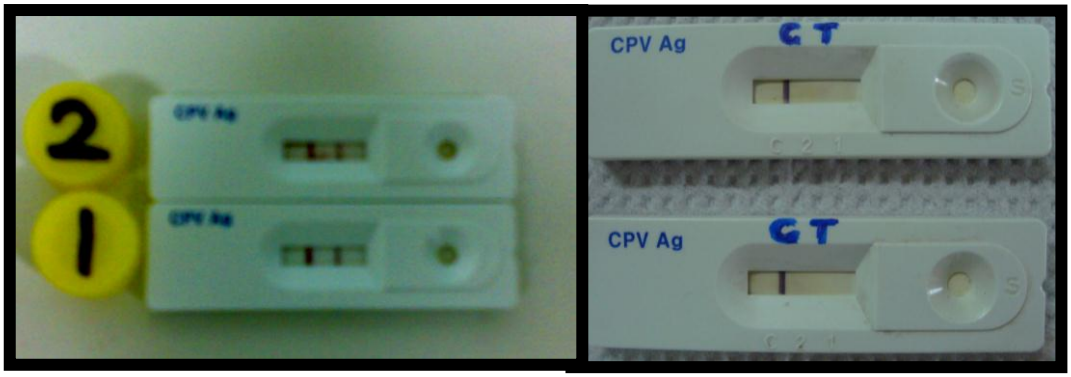
Factor	Condition	Number	%
Age	1-3 months 4-6 months 7-12 months	44 41 5	48.8 45.5 5.5
Breed	Rottweiler Doberman German shepherd	19 9 62	21 10 68.9
Season	September – December 2008 January – May 2009	72 18	80 20
Sex	Female Male	83 7	92.3 7.7

Table (2) Detection of CPV2 antigen by rapid test (RT)

Season	No.of sample	RT positive	RTnegative	% of positive
Sep–Dec 2008 JanMay2009	72 18	44 16	28 2	61 88.9
Total	90	60	30	66.6

Table (3) Show HA titer of positive samples by haemagglutination by using chicken RBCS

HA titer	No. of samples
Less than 16	5
16- 32	24
64- 128	12
256- 526	3
Total	44



A- **B-**

Fig. (1) Rapid test kit for detection CPV antigen (A=positive results, B=negative results) C control, T test

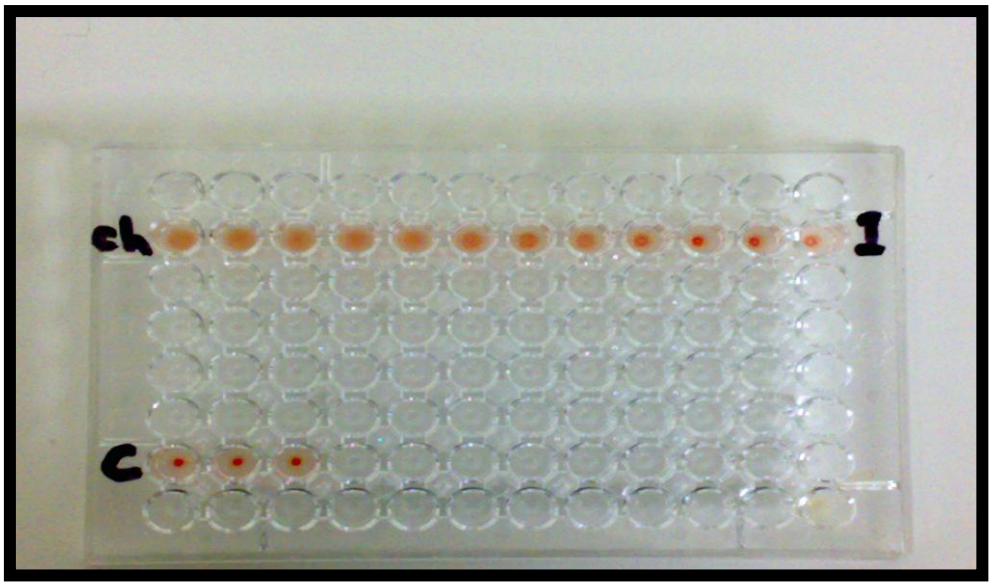


Fig. (2) Haemagglutination test of CPV with chicken RBCs to sample used for isolation

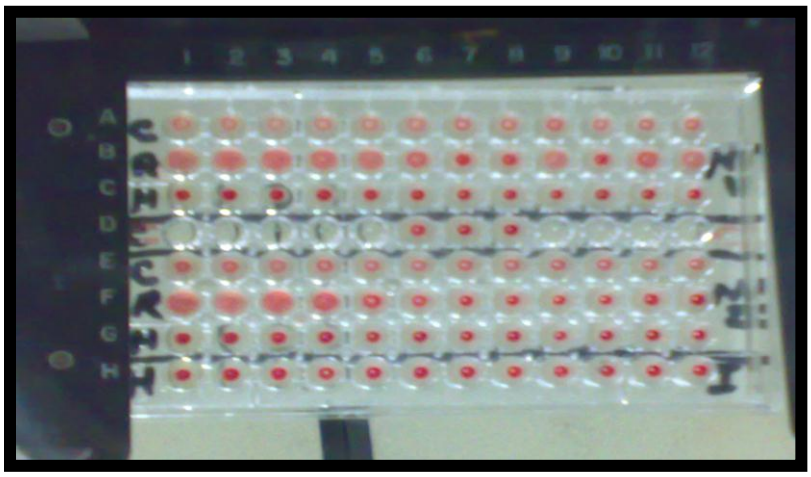


Fig. (3) Haemagglutination test of CPV with canine (C), rabbit(R) and horse (H) RBCs (negative for horse RBCs)

Discussion

CPV2 is considered the most significant cause enteritis of puppies at the age over 2 months of age which cause high mortality rate, and more of cases in our study was recorded at age of 1-6 months Similar observations have been made by (7), who recorded that the disease high risk between weaning to six month of age .The finding also supported by (11) who described the viral replication, had been dependent on the mitotic activity of myocardial and intestinal cells at this age of development, when weaning take place due to lack of maternal immunity and poor immune competency for the acquired immune response at this age could be incriminated to be cause of high incidence of disease. There is few cases at one year similar investigations recorded in (12) this is due to mitotic activity that is restricted to lymphoid organs. The main symptoms of cases bloody diarrhea with or without vomiting and dehydration, (6) have recorded similar observations. Most common breeds infected in our study which were Rotweilers Doberman and Germanshepherd, this result are similar to other study of (13), who recorded the highest incidence of disease in Rottweilers and Doberman. There are different ways for detection of the virus antigen in canine feces one of them is rapid antigen test kit. This enable the specific parvovirus antibodies to identify antigen in canine feces with high degree of accuracy (14). These results agreed with other investigators (15), also same results recorded by (16), when used the same test kit which showed high sensitivity and specifity to detect the subtypes in Iran, also (17) used kit to detect antibodies rather than antigen.

The virus in fecal samples which give positive HA test with avian and mammalian erythrocytes similar observation made by others (18,19,20) Moreover, this could be due to the reason that the avian RBCs are nucleated and heavier and have more density as compared to erythrocytes of other sources, these findings also supported by (21).Also showed there's not agglutination for horse RBCs These results in line with records of (22) who showed that the agglutination of horse RBCs are inconsistence . These results are in line with (1), who used pig and rhesus monkey erythrocyte for the test(23,24,25,26). This test also used for diagnosis of CPV by (18,19,20,21), these showed HA activity due to CPV,these results corroborate with the statement of (27), that shows fecal material in acute condition had 20,000 HA unit of virus or 10^9 virus particle per gram feces.

The HA could be due to present of hemmagglutinins molecules on virus surface. These molecules being glycoprotein in nature have ability to bind on the surface of the avian and mammalian erythrocyte. more over , there's some of positive samples give low titer or complete loss of HA activity after long period of keeping this due to repeated freezing and thawing of fecal samples this leads to degradation of virus hemmagglutinins which is leads to complete loss of HA activity. (7,28) have reported similar results.

The field isolate gave positive HI test with known positive serum similar result recorded by (18,24,26). That showed the results may be helpful in confirming that the HA activity due to CPV. These results also indicate that there was cross protection between vaccinal strain and samples were used. These results in line with (21), HI test indicated presence of CPV in the fecal samples of diseased dogs by using the specific CPV positive serum prepared in rabbits.

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