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Detection of canine distemper virus in stray and pet dogs in Mosul city, Iraq

M.M. Saeed and Q.T. Al-Obaidi

Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

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Correspondence:
Q.T. Al-Obaidi
qaestalb1976@uomosul.edu.iq

Abstract

The current work was carried out during the period from September, 2020 to March, 2021 in Mosul city, Iraq, with the objectives to detection of canine distemper virus (CDV) for the first time in Mosul using microscopic examination of blood smears, rapid serum antigen test and sandwich ELISA test as well as determine the agreement between the different diagnostic methods. A total of 92 blood samples were collected from suspected dogs with CDV (69 stray dogs and 23 Pet dogs). A primarily detection of CDV in blood smear by the presence of inclusion bodies in erythrocytes and leukocytes, followed by detection of CDV antigen in serum using rapid test and sandwich ELISA test. Results indicate that the infection rates of CDV in dogs were 32.6%, 13%, and 19.5% using microscopic examination, rapid test and sandwich ELISA test respectively. A statistically significant higher infection rate was reported in stray dogs compared to pet dogs based on all diagnostic tests used in this study. Based on Kappa values 0.413, 0.675, 0.745, there were moderate agreement between microscopic examination and rapid test, and substantial agreement between microscopic examination and sandwich ELISA test, also between rapid test and sandwich ELISA test respectively. In conclusions, CDV is widespread in dogs in Mosul city, Iraq and all tests used in this study are efficient for detection CDV based on compatibility between them.

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Introduction

Dogs breeding and its diseases is a part of veterinary medicine that is constantly evolving and needs a special approach due to the lack of the important information such as the prevalence of different diseases and the fundamental data in this field especially in Iraq (1). Canine distemper disease (CDD) is a highly contagious, infectious and acutely febrile disease in Canidae (Dogs, Foxes, Wolves, Raccoon), with a worldwide distribution (2). Reports indicate that the disease is the second most dangerous killer of dogs after rabies (3). The disease is caused by a virus belongs to single-stranded RNA virus of the family Paramyxovirade and Morbillivirus genus (4). This virus is antigenically similar to the other members of the Morbillivirus genus, includes measles virus (MV) in humans, rinderpest virus (RPV) in cattle, peste des petits ruminant's virus (PPRV) in sheep and goats, phocine distemper virus (PDV) in seal, dolphin morbillivirus (DMV) in dolphin and porpoise morbillivirus (PMV) in porpoise (2). Canine distemper virus can be transmitted mainly through air droplets contaminated with the secretions of infected dogs, through direct contact between a healthy animal and the secretions of infected dogs, including nasal secretions, saliva, blood and urine, and the virus does not live for long periods in the environment, but it can be transmitted easily through pots fomites contaminated with these secretions (5). In addition, transplacental transmission in dogs but it is not common way of spreading the infection (6). Dogs infected with

canine distemper suffer from respiratory and/ or gastrointestinal signs with or without neurological signs, immunosuppression and skin lesions also can be seen (7). Deaths due to the CDD depend on the virulence of the virus and the specific characteristics of the host, including age, immune status and vaccination status (2), The mortality rate in adult dogs is 50% and in infected puppies it is 80% (8). Diagnosis of CDD is not mainly based on the history of immunization against the disease, the distinctive signs appearing on the affected dogs and the pathological lesions in the dead animals. However, due to suspicion with other infectious diseases agents like canine parvovirus-2, canine alphaherpesvirus-1, canine adenovirus 1 and 2 (9,10), *Hammondia heydorni* (11), *Escherichia coli* (12), *Cryptosporidium spp.* and *Giardia spp.* (13). Therefore, the main laboratory methods to diagnose the disease are isolation of the virus from the nose, conjunctiva or spinal cord fluid (14), serological tests, such as indirect enzyme immunosorbent assay (ELISA), Competitive enzyme immunosorbent assay (ELISA), sandwich ELISA (15), and advances molecular techniques such as the reverse transcription polymerase chain reaction (RT-PCR) and reverse transcription loop-mediated isothermal amplification (RT-LAMP) (16,17). Despite the fact that canine distemper has been clinically diagnosed in dogs at a private practice in Baghdad, Iraq, between 2015-2016 (1).

Therefore, this study was conducted to detection of canine distemper virus (CDV) for the first time in Mosul city, Iraq using different diagnostic methods and to determine the agreement between these methods.

Material and methods

Ethical approval

The study has been approved by the Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

Animals and samples collection

The study was conducted on 92 dogs (69 stray dogs and 23 Pet dogs), from both sexes and different breeds (Local, German and Husky) with ages ranging between 2 months - 1.5 years. The selection of dogs was based on history and suspected clinical manifestations of CDD which include fever, lethargy, oculonasal discharges, coughing, vomiting, diarrhea, dehydration, thickening of the skin of foot pad and around the nose and nervous signs. Dogs were brought to the veterinary teaching hospital, some private veterinary clinics and from different villages of the Mosul city, Iraq, during the period from September, 2020 to March, 2021.

Ninety-two blood samples were withdrawn from all dogs via the cephalic vein and dispended into two tubes, one with ethylenediaminetetraacetic acid (EDTA) anticoagulant for blood smears preparation to detect CDV inclusion bodies inside the erythrocytes and leukocytes, the other tube was plane tube for separating of serum using a centrifuge at 2500 rpm for 10 minutes, then stored -20°C until used.

Microscopic examination of blood smears

Ninety-two thin and thick blood smears were prepared from blood samples were stained with MGG-Quick stain (Bio-Optic, Italy), then examined under the microscope light to detect CDV inclusion bodies inside the erythrocytes and leukocytes (18).

Immunochromatography test (Rapid test)

This test was used as a preliminary and rapid test for detection of antigen specific to canine distemper virus in serum samples using an immunochromatography test kit supplied by Elabscience® Biotechnology Inc. USA. The test was performed according to manufacturer instructions.

Sandwich enzyme linked immunosorbent assay

This assay was used as confirmatory test to detect the antigen of canine distemper virus in serum samples using Canine distemper virus ELISA test kit provided by the American company (Abbexa LLc, USA). The test was performed according to manufacturer instructions.

Statistical analysis

The results were analyzed using the statistical program IBM-SPSS version 22. Using two-sides chi-square test, and Fischer's test to evaluate the difference in the infection rates between pet and stray dogs base on different diagnostic methods. In addition, using the Kappa value to determine the agreement between the different methods used in this study (19).

Results

The results based on microscopic examination of 92 thick and thin smears showed different forms of CDV inclusion bodies inside the erythrocytes and neutrophils (Figure 1), with the infection rate of 32.6% (Table 1).

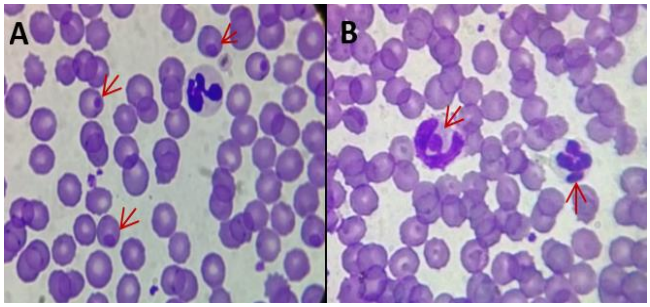

Figure 1: Inclusion bodies of CDV in blood smears stained with MGG-Quick stain, (A): Inside the erythrocyte 1000X, (B): Inside neutrophils 1000X.

In addition, the results of the examination of 92 serum samples of dogs revealed that the infection rates were 13% and 19.5%, based on rapid test and sandwich ELISA test respectively (Table 1).

The results also showed that the infection rate of CDV in stray dogs was 40.5% and 14.5% and 24.6% using microscopy examination, rapid test and sandwich ELISA test respectively.

While, the infection rate of CDV in pet dogs was 8.6%, 0.0%, and 4.34% using the same tests respectively. This indicate that the infection rate of CDV in stray dogs was significantly higher than in pet dogs (Table 2).

The results of this study showed a moderate agreement between microscopy examination and the rapid test in diagnosing of CDV based on the Kappa value, which was 0.413 (Table 3). Moreover, there is a substantial agreement between the microscopic examination and sandwich ELISA test and between the rapid test and the sandwich ELISA test in diagnosing CDV, based on the Kappa value, which was 0.675 and 0.745, respectively, these indicates that all these tests are efficient for diagnose the disease (Tables 4 and 5).

Table 1: The infection rate of canine distemper virus in dogs with different diagnostic techniques

Type of test	No. +ve	Percentage
Microscopic	30	32.6
Rapid test	12	13
ELISA test	18	19.5

Table 2: The infection rate of canine distemper virus in dog with different diagnostic techniques

Type of dogs	No. samples	Type of tests [no. +ve(%)]		
		Microscopic examination	Rapid test	Sandwich ELISA test
Stray dogs	69	28(40.5)a	12(14.5)a	17(24.6)a
Pet dogs	23	2(8.6)b	0(0.0)b	1(3.34)b

Significantly different P<0.05 value is labeled with different superscript letters a or b.

Table 3: Agreement between microscopic examination and rapid test base on kappa value for diagnosis of CDD

		Microscopic examination		
		Infected	Uninfected	Total No.
Rapid test	Infected	11	1*	12
	Uninfected	19**	61	80
	Total	30	62	92

* false positive. ** false negative. Kappa value was 0.413.

Table 4: Agreement between microscopic examination and ELISA test base on kappa value for diagnosis of CDD

		Microscopic examination		
		Infected	Uninfected	Total No.
ELISA test	Infected	18	0*	18
	Uninfected	12**	62	74
	Total	30	62	92

* false positive. ** false negative. Kappa value was 0.675.

Table 5: Agreement between rapid test and sandwich ELISA test base on kappa value for diagnosis of CDD

		Rapid test		
		Infected	Uninfected	Total No.
ELISA test	Infected	12	6*	18
	Uninfected	0**	74	74
	Total	12	80	92

* false positive. ** false negative. Kappa value was 0.745.

Discussion

CDD is over all world distribution with highly morbidity/mortality in spite of vaccinated animals and has no specific treatment (7). In this study different forms of CDV inclusion bodies were detected within erythrocytes and neutrophils based on microscopic examination of blood smears. This finding agrees with result of Da Silva *et al.* (20) Who observed that the presence of CDV inclusion bodies in whole blood confirms evidence in dogs with clinical distemper. Earlier study reported this result as uncommon in a blood film (21).

The outcomes of the present study, based on laboratory examination of 92 blood smears and serum samples using microscopy examination, rapid test and sandwich ELISA test, showed that the infection rates of CDV in dogs in Mosul city were 32.6%,13% and 19.5% respectively. There are numerous studies which detected the prevalence of CDV in different countries. In Turkey, 94% IgG and 58% IgM positive samples were detected by the CDV-specific indirect ELISA in the 50 serum samples (7), in Iran was 17.52% using IFA (22), in Haa, Western Bhutan was 11.3% using ELISA test (15), and in Mizoram, India was as1.11% using the antigen rapid CD virus Ag test kit (23). Moreover, in Brazil, the detection rate of CDV in urine samples was 100% in symptomatic dogs tested using One-Step RT-qPCR (24). The prevalence of CDD in different studies and different areas varies and may be related to the percentage of specificity of diagnostic method, stage of CD present and the vaccination status of dogs (25).

This study indicates that the infection rate of CDV in stray dogs was significantly higher than in pet dogs. This finding agrees with the result of Swapna *et al.* (26) They mention that higher prevalence of CDV in stray dogs which were 9.03% and 4% respectively. In addition, Acosta-Jamett *et al.* (27) revealed that a maintained canine population management and pet possession are the only arrangements to reduce canine population, which would, in turn, reduce canine-wildlife interactions and outcomes pathogen spreading. On the other hand, Saltik and Kale, (7) and Dorji *et al.* (15) mentioned that no significant difference in the prevalence of CDD between stray and pet dogs. In particular, vaccination programmes for pet dogs are more used in private veterinary clinics. Nevertheless, since no vaccination programme is applied to stray dogs, all the vaccinated or unvaccinated dogs are at risk of the disease (28). This study revealed that infected dogs with CDD showed fever, lethargy, eye and nasal discharges, coughing, vomiting, diarrhea, dehydration, thickening of the skin of foot pad and around the nose and nervous signs, these signs were the similar to those reported by Saltik and Kale, (7) and Amude *et al.* (29).

This is the first study showed the compatibility between the different methods (Microscopy examination, rapid test, and sandwich ELISA test), for detection CDV based on Kappa value which were 0.413, 0.675 and 0.745. This indicates that all these tests are efficient for diagnose the disease. Da Silva *et al.* (20) indicate that microscopic examination of blood smears for detection CDV inclusions confirm the occurrence of the disease in dogs with clinical manifestations. The rapid CDV Ag test kit can be used for the detection of CDV in various dog samples includes nasal fluid, saliva, conjunctival secretion, serum and urine (23). Furthermore, a ELISA is sensitive, specific, and simplify assay with good reliability for detection of CDV antigen that could be suitable for high-throughput testing applications (30).

Conclusion

Our study indicates that CDD is widely distributed among stray and pet dogs, with significantly higher in stray dogs in Mosul city, Iraq. It can be diagnosed by clinical signs combined with rapid test and ELISA. Moreover, we propose reinforcement the management practices of dogs through dependable dog proprietorship, and apply strategically programme for vaccination of both stray and pet dogs against CDV as a control of disease.

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Conflict of interest

The authors declare no conflicts of interest.

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الكشف عن فيروس طاعون الكلاب في الكلاب السائبة والأليفة في مدينة الموصل، العراق

مهند مروان سعيد و قيس طالب العبيدي

فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تم إجراء العمل الحالي خلال الفترة من أيلول ٢٠٢٠ إلى آذار ٢٠٢١، في مدينة الموصل، العراق للكشف ولأول مرة عن فيروس طاعون الكلاب باستخدام الفحص المجهري لمسحات الدم واختبار مستضد المصل السريع واختبار الممتز المناعي الساندويج. كما تم تحديد التوافق بين تلك الطرق التشخيصية المختلفة. تم جمع ٩٢ عينة دم من الكلاب (٦٩ كلبا سائبا و ٢٣ كلبا أليفا)، اشتبه سريريًا بإصابتها بطاعون الكلاب. تم الكشف الأولي عن الأجسام الاشتمالية لفيروس طاعون الكلاب في كريات الدم الحمراء وخلايا الدم البيضاء اعتمادا على الفحص المجهري لمسحات الدم، كما تم الكشف عن مستضد طاعون الكلاب في المصل باستخدام الاختبار السريع واختبار الممتز المناعي الساندويج. أظهرت النتائج أن معدلات الإصابة بفيروس طاعون الكلاب بلغت ٣٢,٦، ١٣ و ١٩,٥٪ باستخدام الفحص المجهري والاختبار السريع واختبار الممتز المناعي الساندويج على التوالي. ولوحظ ارتفاع معنوي في معدل الإصابة في الكلاب السائبة مقارنة بالكلاب الأليفة اعتمادا على جميع الاختبارات التشخيصية في هذه الدراسة. واستنادًا إلى قيم كابا ٠,٤١٣، ٠,٦٧٥ و ٠,٧٤٥، كان هناك توافق متوسط بين الفحص المجهري والاختبار السريع، وتوافق حقيقي بين الفحص المجهري واختبار الممتز المناعي، وأيضًا بين الاختبار السريع واختبار الممتز المناعي على التوالي. استنتجه من هذه الدراسة الأولى من نوعها، إلى انتشار فيروس طاعون الكلاب في الكلاب في مدينة الموصل، العراق وان جميع الاختبارات المستخدمة في هذه الدراسة ذات فاعلية في الكشف عن المرض على أساس التوافق بينها.

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