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Molecular investigation of feline calicivirus in cats in Mosul city, Iraq

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Abstract

The study included 200 cats of different ages, genders, types of breeding, source, vaccination programs, and health status. They were examined clinically and traditionally and recorded clinical signs appearing on them, and swabs were collected from the eye's conjunctiva and the pharyngeal area. RNA was extracted from the swabs and then converted into a cDNA molecule to investigate viral nucleic acid from collected swabs. Then, the open reading template gene two was detected using the primer for this within the applied polymerase chain reaction technique. The Molecular method found the highest infection rate in the oropharyngeal compared with conjunctival swabs. It was found in the highest percentage of infection in the age group more than six months, and the rate of infection decreased with age and in cats outdoors management and imported ones. While nonvaccinated cats recorded the highest rate of infection with the feline calicivirus. In conclusion, feline calicivirus affected the cats in Mosul, Iraq.

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Introduction

Feline calicivirus is one of the primary pathogens of respiratory infection in cats. The symptoms vary depending on the virulence of the virus strain, the immune status of the animal, age, source of the animal, and other environmental factors epidemiological factors (1,2). Feline calicivirus is a member of the Caliciviridae family, genus vesivirus, which possesses positive-stranded RNA (3). Calicivirus achieved the name from Latin "calyx," which means cup-like depressions on the capsid. Another character of this virus is the high mutation rates due to mismatched nucleotides copied, which are repaired during translation (4). The variability and diversity of FCV strain resulted in variation in clinical signs such as upper respiratory tract infection (5), lameness, stomatitis, and systemic clinical signs, the typical clinical signs manifested by nasal and oral discharge, congestion in the mucus membrane, pharyngitis, ulceration in mouth mucosa, pneumonia, fetal death, abortion, edema in head and joints, voice loss, lameness, fever, depression, sneezing, jaundice, and enteritis (6). After recovery from feline calicivirus infection, cats persistently shed the virus from the oro-pharynx for up to 30 months with a high titer of antibodies. High-level of shed virus in carrier's animals are detectable easily and depends on the individual suitability of cats to infection, while common virus shedding in carriers is difficult to detect and requires several attempts (7). The virus is transmitted directly by contact with infected cats or indirectly through contaminated fomites in which the virus survives on fomites for up to 3 weeks in dried conditions (8). Diagnosis of FCV is achieved by virus isolation in Crandel Reese feline kidney (CRFK) cells, serological identification by immunofluorescence (IFAT), neutralization test, and molecular identification by Reverse-transcription Polymerase Chain Reaction (rt-PCR) using a primer to a segment of the capsid protein gene (9). The Reverse-transcription Polymerase Chain Reaction is an advantageous method as it is accessible, rapidly sensitive. For those reasons, FCV-specific RT-PCR assay has been developed and widely used to detect FCV infection (10). However, the high degree of gene variation in FCV genome features reduces its sensitivity. As gene mutations occur, the

nucleotide sequences of primer binding sites are likely to be changed (11).

Because of high incidence rates and prevalence despite FCV vaccination of cats, the current study is the first trial for molecular detection of FCV in Iraq.

Materials and methods

Swabs collection

Two hundred swabs were collected from the conjunctiva and oropharynx of cats at different ages, breeding management, health status, vaccination history, and source. Samples were transferred to Veterinary teaching hospitals, the University of Mosul, and 5 Veterinary clinics for pet animals. Each swab was suspended in 1 ml of sterile phosphate-buffered saline (PBS), then centrifuged at 3000 rpm for minutes at 4ºC, the sediments were discarded, and the suspension was collected for molecular assay (12).

Molecular assay

Reverse transcriptase PCR was carried out according to manufacturer instructions. cDNA was synthesized from the extracted RNA using RNA to cDNA Eco Dry Premix (Clontech, Japan, Cat No. 639546). The forward primer, ORF2-F (5′-CTGCCTCCTACATGGGAAT-3′), binds on region F of ORF2, of FCV strain F9. The binding site of reverse primer, ORF3-R (5′-GTGTATGAGTAAGGGTCRACCC-3′), (13) is located on the starting region of ORF3. The expected size of the target amplicon was calculated as 324 bps. The PCR reaction was performed in a 20 µL mixture containing two µL of cDNA solution, one µL of each primer (10 pmol), 2.5 mM of each dNTP, ten mMTris-HCL (pH 8.8), 1.5 mM MgCl2, 50 mMKCl, and 2.5 U of Taq polymerase. Thermal cycling conditions consisted of denaturation (95ºC, 5 min), followed by 35 cycles of denaturation (95ºC, 30 sec), primer annealing (57ºC, 30 sec), and primer extension (72ºC, 30 sec). A final extension was performed at 72ºC (5 min). Any swab gives a positive result; the animal is considered positive for infection with the FCV.

Statistical analysis

The difference in infection percentages between the different ages, source, breeding management, vaccination status of cats was assessed using a two-sided Chi-square test in the IBM-SPSS statistics version 19 program (14).

Results

Two hundred conjunctival and oropharyngeal swabs tested using rt PCR revealed differences according to the swab method, as the oropharyngeal swabs showed a high rate of infection compared to swabs taken from the conjunctiva (Table 1, Figure 1). According to age, the kitten up to six months showed a high prevalence of infection with FCV, while cats for more than one year recorded a low prevalence of infection, which is statistically significant (Table 2). According to breeding management, outdoor animals have a higher prevalence than outdoor animals without significant differences (Table 3). The results showed high prevalence in the imported animal while the native animals gave lower infection prevalence without significant difference (Table 4). Non-vaccinated animals showed a high prevalence of infection, while the vaccinated animals showed a lower prevalence of infection without significant difference (Table 5).

Table 1: Percentage of infection with FCV in different types of swabs

Source of swab	No sample	No +ve	% Infection
Oropharyngeal	200	89	44.5
Conjunctival	200	37	18.5

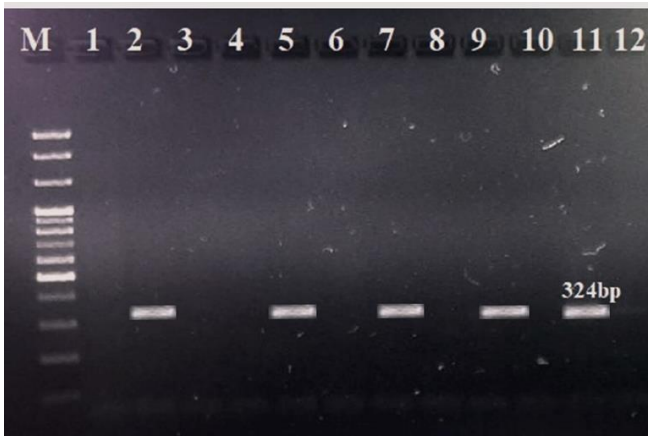

Figure-1- results of electrophoresis of final products of rtPCR at 324bp, M=Marker, 1, 3, 4, 6, 8, 10=negative results, 2, 5, 7, 9=positive results, 11=positive control (F9 strain), 12=Negative control.

Table 2: Percentage of infection with FCV according to the age of cats

Age (month)	No sample	No +ve	% Infection
> 6	87	41	47.1[a]
6 to 12	59	29	49.1[a]
<12	54	19	35.1[b]
Total	200	89	44.5

Values significantly different (P<0.05) between animal ages are labeled with superscript letters (a, b).

Table 3: Percentage of infection with FCV according to management

Breeding	No sample	No +ve	% Infection
Indoor	134	61	45.5[a]
Outdoor	57	28	49.1[a]
Total	200	89	

[a] There is no significant differences between different breeding management.

Table 4: Percentage of infection with FCV according to the source of cats

Source	No sample	No +ve	% Infection
Imported	96	47	48.9[a]
Native	104	42	40.3[a]
Total	200	89	

[a] There is no significant differences between the source of animals.

Table 5: Percentage of infection with FCV according to vaccination history of cats

Vaccination	No sample	No +ve	% Infection
Vaccinated	144	48	33.4[a]
Non vaccinated	56	41	73.2[b]
Total	200	89	

Values significantly different ($P<0.05$) between vaccination history are labeled with superscript letters (a,b).

Discussion

Feline calicivirus (FCV) is a ubiquitous respiratory and oral pathogen of domestic cats that infects cats of all ages, especially kittens (14). The results of conventional rt-PCR revealed differences between the presence of virus nucleic acid in the oropharyngeal and conjunctival swabs, which were elevated in oropharyngeal swabs compared to conjunctival swabs. When comparing these results with other studies, it turns out that many concepts are proving this, some of which agree with our results, and others contradict them. The researchers (15) stated that the sites and mechanism of persistent infection with feline calicivirus are epithelial cells in other oral and pharyngeal tissues in infected cats. In addition, these tissues provide some protection for the virus from the immune system, allowing it to replicate in it, but with low titers (16). Another reason for the differences is the variation in shedding patterns of feline calicivirus during the sampling period, as the virus shed is relatively steadily over long periods (consistent shedding), while some virus strains shed intermittently (intermittent shedding), as they do not shed the virus all the time (17). At the same time, our results differ from others (18), which indicated high rates in swabs taken from the conjunctiva compared to swabs taken from the mouth, pharynx, and nose. At the same time, the researchers (14) indicated that the infection rate was 45% in the oral, pharyngeal, nasal, and conjunctival swabs samples, which were collected from 200 cats with upper respiratory diseases and 19 different regions of Switzerland using the polymerase chain reaction technique. While a different pattern of infection with the feline calicivirus appeared by researchers (19), who found that there were low rates of infection from swabs collected from the mouth with a low standard of viral particles, and the reason was attributed to the presence of ribonuclease enzyme in the oral mucosa secretions as well as a genetic variation of the virus.

The polymerase chain reaction results showed a decrease in the incidence of feline calicivirus with increasing age. It agrees with the results mentioned by the researchers (20), where they indicated that the infection of the virus increases with age, as the infection rate was 7% in the ages less than 11 years, while in ages 1-3 years, the infection rate was 7.4%, and the results of the researchers (21) also agreed with our study, where its results showed that infection with the virus was high in the ages less than six months. Such differences could be due to several reasons, including that the mechanism of the immune response to acquire resistance against infection in older cats (more than three years) was more significant compared to younger cats, which affects the criterion of the virus that is released outside the body, which in turn affects the criterion of the virus present in the sample. Another reason is that older cats are exposed to more virus vaccinations compared to younger cats that were primarily vaccinated once, which leads to older animals having a better immune response compared to younger animals (22).

The study showed high rates of infection in animals raised indoors compared to cats raised outside homes. One study indicated the nature of breeding in the rate of infection with feline calicivirus, as it indicated that increasing the population density, regardless of the type of breeding system, increases the chances of infection with the virus (23). At the same time, another study showed an association between the introduction of new cats in the house and an increase in the incidence of the virus (24).

This study showed high infection rates in imported cats compared to native cats. One study (14) indicated differences between the breed of cats and the sensitivity of infection with the feline calicivirus. Other explanations may include the stress factors as imported cats are exposed during transportation and the differences between virus strains in different countries. In addition, it has been recently shown that the virus junctional adhesion molecules 1 (JAM -1), which are receptors for the virus shapes and diversity, have a significant role in determining whether cats are resistant or sensitive to infection, which in general is determined by the source of animals (25). In addition, the imported cats to the

city of Mosul are often young or may not exceed the age of weaning, making them sensitive to infection (researcher field observations).

The study showed high infection rates in vaccinated animals, while non-vaccinated animals showed a lower percentage of infection without significant differences. Several studies indicated that vaccination does not protect cats from reinfection with feline calicivirus but instead reduces the severity of clinical signs only (26). So, it is clear why vaccination did not reduce the chance of carrying and shedding feline calicivirus in this study and others, and this is what this study confirmed it, as many of the vaccinated cats that gave positive results for the polymerase chain reaction gave positive results at the same time for the immunofluorescence test, which detects the virus-specific antibodies (27). It was found that the rate of infection with feline calicivirus in vaccinated cats was high at 23.08% compared to unvaccinated cats at a rate of 19.23% using semi-nested polymerase chain reaction technique. These results were explained by the presence of genetic variation of the virus between virulent fields and vaccinated strains, while researchers (28) indicated the presence of infection with the virus.

Conclusions

Feline calicivirus was distributed in cats in Mosul city and affected cats at different ages, vaccination status, and breeding management.

Acknowledgment

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Conflict of interest

The authors declare no conflict of interest in the manuscript.

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التقصي الجزيئي عن الفيروس الكأسي السنوري في القطط في مدينة الموصل

الاء كاظم حمدان و صفوان يوسف الباروودي

فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

شملت الدراسة ٢٠٠ قطاً بمختلف الأعمار، الأجناس، طبيعة التربية، المصدر، وبرامجها التحصينية فضلا عن الحالة الصحية، وتم فحصها سريريا وبالطرق التقليدية وسجلت العلامات السريرية الظاهرة عليها، ومن ثم جمعت منها مسحات من ملتحمة العين ومنطقة البلعوم ولغرض التقصي عن جزيئة الرنا للفايروس الكاسي السنوري من المسحات التي تم جمعها خلال الدراسة من خلال استخلاص الرنا من المسحات ومن ثم الكشف عن جين قالب القراءة المفتوحة ٢ من خلال استخدام البادئ الخاص بذلك ضمن تقنية تفاعل البلمرة المتسلسل الذي تم تطبيقه على الحامض النووي المستخلص لكل عينة، بينت نتائج الدراسة الجزيئية وجود اعلى نسبة إصابة بالفايروس الكاسي السنوري من المسحات التي جمعها من منطقة البلعوم مقارنة بالمسحات التي جمعها من ملتحمة العين، كذلك تبين أن اعلى نسبة إصابة كانت في الفئة العمرية الاكبر من ٦ اشهر وانخفضت نسبة الإصابة بتقدم العمر كذلك سجلت اعلى نسبة إصابة بينت نتائج الدراسة الجزيئية بان اعلى نسبة إصابة كانت في القطط التي تربى خارج المنازل فضلا عن المستوردة منها، في حين سجلت القطط الغير المحصنة اعلى نسبة إصابة بالفايروس الكاسي السنوري.

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