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RESEARCH ARTICLE

Serology-Based Approach in the Clinical Evaluation of Neonatal Viral Eye Diseases in Kittens: Calicivirus, Herpesvirus and Panleukopenia Virus

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Abstract: This study aimed to examine the distribution of feline calicivirus (FCV), feline herpesvirus (FHV), and feline panleukopenia virus (FPLV), which cause neonatal feline viral diseases in kittens aged one to three months, using a Dot-ELISA bases antibody test kit. The studied parameters included the animals' sex, clinical signs and the Dot-ELISA test kit values. Twenty kittens had eye lesions and 20 were without eye lesions. Basic ophthalmologic examinations were performed, including pupil, corneal, palpebral and menace response reflexes, direct ophthalmoscopy, and fluorescein staining. The study population consisted of 40 kittens (25 female and 15 male); 3 of them are British shorthaired and the rest is 37 tabby kittens. In half of the 20 kittens with eye lesions, the lesions were bilateral and the most common clinical lesions were conjunctivitis, mucopurulent discharge, and blepharospasm. Other notable clinical findings were iris staphyloma, corneal opacity, symblepharon, and panophthalmitis. A higher rate of seropositive results was determined against Calicivirus in kittens. The severity and appearance of the cases could vary depending on the virus accompanying the lesions. In conclusion, the Feline Calicivirus was the most frequently detected virus in 1 to 3-month-old kittens in this study and the clinical presentation may change according to the accompanying virus titers.

Keywords: *Dot-ELISA, Eye disease, Kitten, Newborn, Viral infection*

Yavru Kedilerde Neonatal Viral Göz Hastalıklarının Klinik Değerlendirmesinde Seroloji Temelli Yaklaşım: Calicivirus, Herpesvirus ve Panleukopenia Virus

Özet: Bu çalışmada, 1-3 aylık yavru kedilerde neonatal kedi viral hastalıklarına neden olan feline calicivirus (FCV), feline herpesvirus (FHV) ve feline panleukopenia virus (FPLV) dağılımlarının Dot-ELISA bazlı antikor testi kullanılarak incelenmesi amaçlanmıştır. İncelenen parametreler hayvanların cinsiyetini, klinik belirtilerini ve Dot-ELISA test kiti değerlerini içeriyordu. Yirmi yavru kedide göz lezyonu vardı ve 20'sinde göz lezyonu yoktu. Pupil, kornea, palpebral ve tehdit tepki refleksleri, direkt oftalmoskopi ve floresein boyama dahil olmak üzere temel oftalmolojik muayeneler yapıldı.Çalışma popülasyonu 40 yavru kediden (25 dişi ve 15 erkek) oluştu. Bunlardan 3'ü kısa tüylü İngiliz, geri kalanı 37 tekir kedi yavrusuydu. Göz lezyonu olan 20 yavru kedinin yarısında lezyonlar bilateraldi ve en sık görülen klinik lezyonlar konjonktivit, mukopurulent akıntı ve blefarospazmdı. Diğer dikkate değer klinik bulgular ise iris stafilomu, korneal opasite, simblefaron ve panoftalmitisdi. Yavru kedilerde Calicivirus'a karşı daha yüksek oranda seropozitif sonuç belirlendi. Olguların şiddeti ve görünümü lezyonlara eşlik eden virüse göre değişebilmektedir. Sonuç olarak, bu çalışmada 1-3 aylık yavru kedilerde Feline Calicivirus en sık saptanan virüs olmuştur ve klinik tablo eşlik eden virüs titrelerine göre değişebilmektedir.

Anahtar sözcükler: *Dot-ELISA, Göz hastalığı, Yavru kedi, Yenidoğan, Viral enfeksiyon*

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Introduction

In kittens, eyes open at about 10-14 days old. Premature opening of the eyes rarely occurs in the kitten, but it can cause corneal drying, keratitis, corneal ulcers and conjunctivitis. More common is the delayed opening of the eyelids which is referred to as ankyloblepharon. Ankyloblepharon is usually caused by mucus accumulation and sometimes due to infection *(ophthalmia neonatorum)* [1]. The main viral infectious agents that cause neonatal eye infections in cats include feline herpesvirus (FHV), feline calicivirus (FCV), and less commonly feline panleukopenia virus (FPLV) [2,3].

FHV is one of the main causes of conjunctivitis in kittens. Primary symptoms seen in kittens include fever, sneezing or coughing, rhinitis, nasal discharge, and conjunctivitis. Transmission of FHV-induced neonatal ophthalmia can occur from mother to kitten or shortly after birth. It occurs in kittens, especially in the 8-12 week age range when maternal antibodies are reduced [4]. The cytopathic virus causes severe ulceration of mucosal surfaces and serosanguineous ocular or nasal discharge. Affecting the substantia propria of the conjunctiva and the stroma of the cornea, symblepharon or adhesion may occur between these tissues. In a viral infection, large amounts of inflammatory debris may accumulate in the conjunctival sac *(conjunctivitis neonatorum)* [2].

Feline calicivirus (FCV) is primarily a viral pathogen of the cat's respiratory tract and can also cause oral ulcers and polyarthritis. FCV can cause severe conjunctivitis in cats but is a less common cause of conjunctivitis than FHV [4]. In a study in cats with ocular surface disease and upper respiratory tract infection, moderate to severe conjunctivitis was seen with conjunctival epithelial erosions in FCV-positive cats [5].

Feline panleukopenia virus (FPLV), a feline Parvoviral pathogen, infects cats mostly up to 1 year of age through intrauterine transmission. Kittens born to infected cats have tremors, ataxia and hypermetric gait, and cerebellar hypoplasia. Ocular symptoms have been reported as retinal dysplasia and degeneration. Conjunctivitis has been reported in two experimentally infected kittens [3,6].

Today, several laboratory methods are used to detect viral antigens or antibodies. Among these, serological methods based on the detection of specific antibodies, which are the host's response to the virus, are the most widely used [3]. Dot-ELISA-based methods are one of the rapid ELISA tests that can be a good option according to previous studies [7,8].

The presented study aimed to detect FCV, FHV and FPLV in kittens presented to our clinic with neonatal ophthalmia symptoms, using a rapid Dot-ELISA test kit and to evaluate them together with clinical symptoms.

Material and Methods

Ethical Statement

The use of the data constituting the study was approved by the Local Ethics Committee (Approval no: 2021/138).

Clinical Examination

The data were obtained from forty kittens divided into 2 groups, 20 patients with various eye complaints and 20 healthy kittens who came for their first examination and vaccinations to the Animal Hospital. All cases underwent basic ophthalmologic examinations, including pupil, corneal, palpebral and menace response reflexes, direct ophthalmoscopy, and fluorescein staining. If deemed necessary in a condition such as corneal edema, hyphema or total symblepharon, vitreous or retinal abnormalities, complementary techniques were performed including ocular ultrasonography and binocular ophthalmoscope.

Diagnostic Analysis

Blood samples were collected from kittens into serum separator tubes (SST). Sera were separated at 1500 g x 10 min after complete clot formation. The ImmunoComb® Feline VacciCheck Antibody Test Kit (Biogal, Kibbutz Galed, Israel), the method principle is Dot-ELISA, is designed to detect serum IgG antibody titers against FCV, FHV and FPLV in kittens. The assay was performed following the manufacturer's instructions. The final step was the development of a grey color tone following sequential washing and the binding of an enzyme-linked anti-cat immunoglobulin G antibody. Positive results were defined as color tones that were equivalent to or darker than the positive control, while negative results were defined as color tones that were paler than the positive control. Besides, the degree of color tones was also evaluated and semi-quantitative titering was done as follows: S0: Negative, S1 or less: Negative; S2: Weak positive, S3 and S4: Positive, S5 or more: Strong positive.

Statistical Analysis

Collected data were organized in Microsoft Office Excel 2010® and the statistical analyses were performed using descriptive statistics with the IBM SPSS Statistics 21® software. The titers of the groups with and without eye lesions were evaluated according to the Wilcoxon test, and the titers between the groups were evaluated with the Mann-Whitney U tests.

Results

The study was performed on 40 kittens, aged 1-3 months (mean±SD 1.9 months). The mean age of 20 kittens with eye lesions was 1.7 months, the mean age of 20 kittens without lesions was 1.9 months, and the average age of all kittens was 1.8 months. Of these 40 kittens, 25 were female and 15 were male.

The most detected virus in this study was FCV. While S3-S4 and above positive results were found in 19 of the kittens with eye lesions, S2-poor positive results were detected in only one kitten *(Table 1)*.

Table 1. *FCV, FHV and FPLV results of each individual kitten*

Kitten Number	Group	FCV	FHV	FPLV
1	Healthy Kitten	S4	S1	S1
2	Kitten with Eye Lesions	S4	S0	S0
3	Kitten with Eye Lesions	S5	S0	S0
4	Kitten with Eye Lesions	S6	S0	S0
5	Healthy Kitten	S4	S2	S2
6	Healthy Kitten	S6	S1	S2
7	Kitten with Eye Lesions	S4	S2	S2
8	Kitten with Eye Lesions	S4	S0	S6
9	Healthy Kitten	S2	S0	S0
10	Healthy Kitten	S5	S0	S2
11	Healthy Kitten	S3	S0	S2
12	Kitten with Eye Lesions	S6	S0	S5
13	Healthy Kitten	S4	S0	S2
14	Kitten with Eye Lesions	S4	S0	S3
15	Healthy Kitten	S5	S0	S3
16	Kitten with Eye Lesions	S6	S2	S0
17	Healthy Kitten	S2	S0	S5
18	Healthy Kitten	S0	S4	S0
19	Kitten with Eye Lesions	S4	S0	S0
20	Healthy Kitten	S0	S4	S0
21	Kitten with Eye Lesions	S4	S2	S2
22	Kitten with Eye Lesions	S4	S0	S1
23	Kitten with Eye Lesions	S4	S0	S2
24	Kitten with Eye Lesions	S4	S0	S3
25	Healthy Kitten	S4	S1	S0
26	Kitten with Eye Lesions	S2	S5	S1
27	Healthy Kitten	S6	S1	S1
28	Healthy Kitten	S4	S1	S0
29	Kitten with Eye Lesions	S4	S1	S1
30	Kitten with Eye Lesions	S4	S0	S0
31	Kitten with Eye Lesions	S4	S0	S1
32	Healthy Kitten	S1	S0	S0
33	Kitten with Eye Lesions	S4	S0	S3
34	Healthy Kitten	S1	S0	S0
35	Healthy Kitten	S3	S2	S1
36	Kitten with Eye Lesions	S4	S2	S2
37	Kitten with Eye Lesions	S3	S4	S2
38	Healthy Kitten	S2	S1	S1
39	Healthy Kitten	S0	S0	S2
40	Healthy Kitten	S0	S0	S1

In 20 kittens without eye lesions, also FCV was the most detected virus S3-S4 and above positive results in 11, weakly positive results in 3 kittens, while 6 kittens had S1 or less negative results. In kittens without eye lesions, FPLV was found to have positive results of S3-S4 and above in 2 kittens, S2 weakly positive results in 6 kittens, S1 and six negative results in 12 kittens. In FHV, 2 kittens had S3-S4 and above results and 2 with S2 weakly positive results whereas 16 kittens were determined to have S1 and six negative results. However, no significant differences were determined in the intragroup virus titers assessment or the intergroup virus titers assessment of kittens with and without eye lesions *(Fig. 1)*.

Fifteen out of 20 cases with ocular complaints had complaints of not opening the eyelids or being closed due to the discharge. On the other hand, general clinical examination findings included conjunctivitis in 13 kittens, mucopurulent discharge in 7 kittens, corneal opacity in 7 kittens, iris staphyloma in 3 kittens, serous discharge in 2 kittens, panophthalmitis in 2 kittens, keratitis in 2 kittens, corneal edema in 1 kitten and microphthalmia was detected in 1 kitten. Here, conjunctivitis was the most detected lesion with an incidence of 13 kittens *(Fig. 2)*. In 5 kittens, conjunctivitis was observed to be bilateral. In 12 kittens, FCV was the most detected virus, with a positive result of S3-S4 and above. FHV S4 and S5 positive results were determined in 2 cases. FCV was detected in only four cases, 3 of these kittens were siblings *(Fig. 3)*. Again, in one of the conjunctivitis cases, FPLV S5 positive result was obtained in the kitten.

Mucopurulent eye discharge seen in 10 kittens was the second most common clinical sign *(Fig. 4)*. While FCV, S4 and above were positive in all kittens, only FCV was detected in 4 of them. FPLV S3 positive results were determined in 2 of the kittens with conjunctivitis, while S2 and below results were obtained in the other two. On the other hand, serous eye discharge was observed in only 2 kittens and these kittens had FHV-positive results of S4 and above *(Fig. 5)*.

The other most detected lesion was corneal opacity in 7 kittens *(Fig. 2)*. While PCV was positive in these cats, S3-S4 and above in 5 kittens, only FCV S4 was positive in one kitten. Corneal opacity was observed bilaterally in 2 kittens, and FCV S4 was positive in these cats. The other virus-causing corneal opacity was FHV with S4 and S5 positive results *(Fig. 1)*.

Iris staphyloma was the other clinical sign, with FCV-positive results of S4 and above in 3 kittens. In one of these kittens, the case was observed bilaterally with an S6 positive result. The clinical findings observed in kittens with FCV-positive results of S4 and above were symblepharon in 2 kittens, panophthalmitis in 2 kittens, keratitis in 2 kittens,

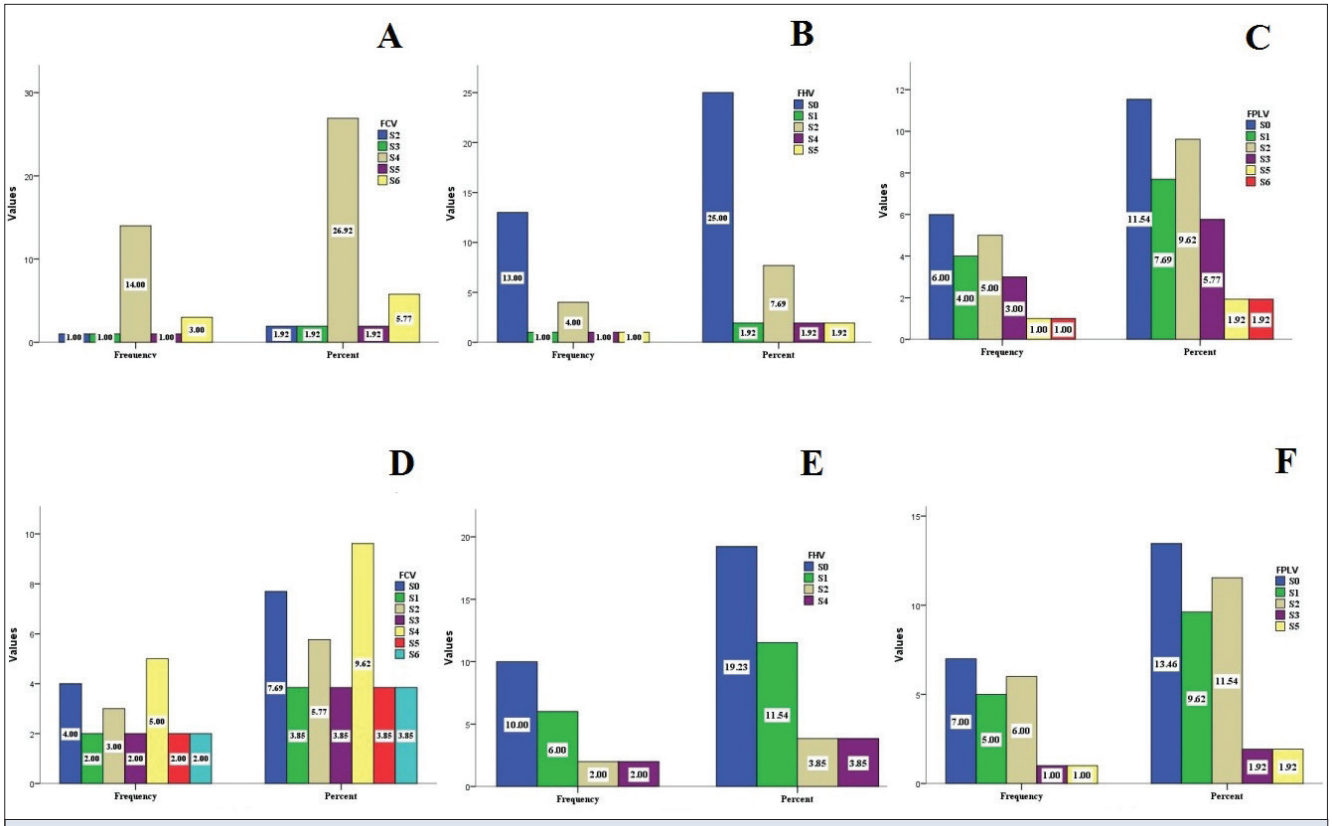

Fig 1. Titers distribution of FCV (A), FHV (B), FPLV (C) in kittens with eye lesions and FCV (D), FHV (E), FPLV (F) in kittens without lesions. FCV was the most detected virus with S4 positive titer in both kittens with eye lesions (14/26.92%) and kittens without lesions (5/9.62%)

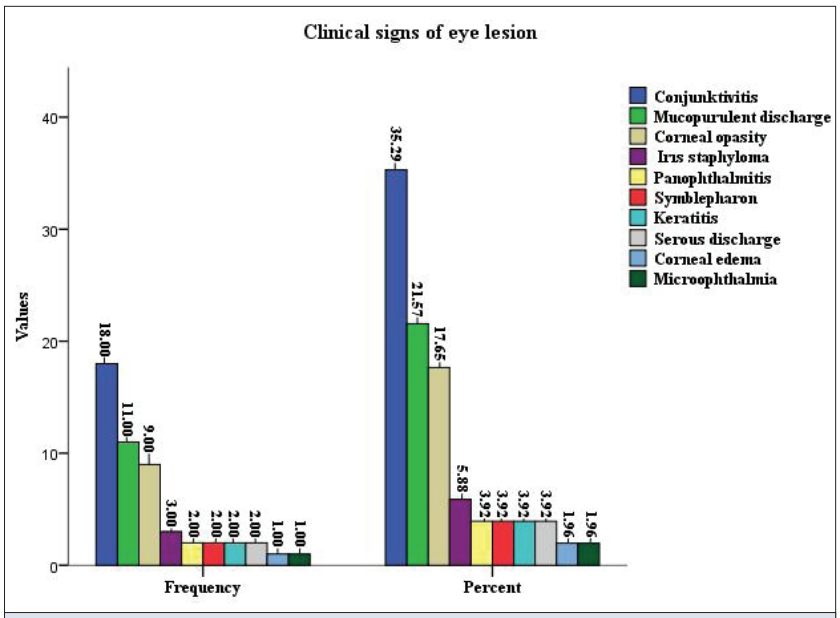

Fig 2. Percentage and frequency of clinical signs. The most common clinical symptom detected in kittens with eye lesions was conjunctivitis (18/35.29%), and the lesion was observed unilaterally in 13 kittens and bilaterally in 5 kittens

corneal edema in 1 kitten, and microphthalmia caused by corneal perforation in 1 kitten *(Fig. 2)*. In one of the keratitis and panophthalmitis cases, FPLV accompanied FCV with a positive result of S5 and above.

The bilateral nasal discharge associated with respiratory system lesions was detected in 15 of the kittens with eye lesions, except for one kitten with unilateral discharge. Fluorescein staining positive results were obtained from

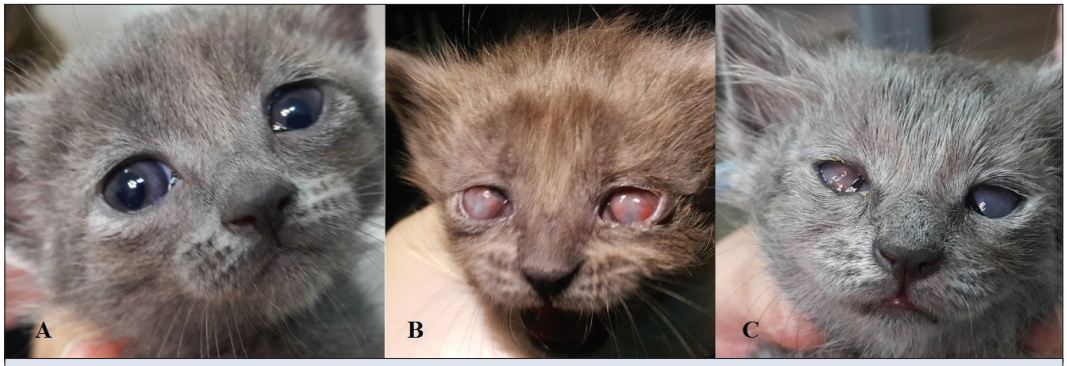

Fig 3. Image of variable lesions, (slight corneal edema (A), severe bilateral keratitis, corneal vascularization and symblepharon (B), mild corneal edema in left eye and partial symblepharon, keratitis and iris stahyloma in right eye (C)) in 3 FCV high positive sibling kittens

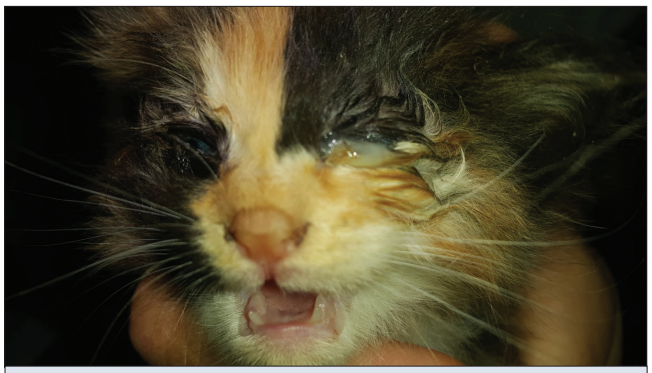

Fig 4. Bilateral ankyloblepheron and mucopurulent ocular discharge in a kitten with FCV

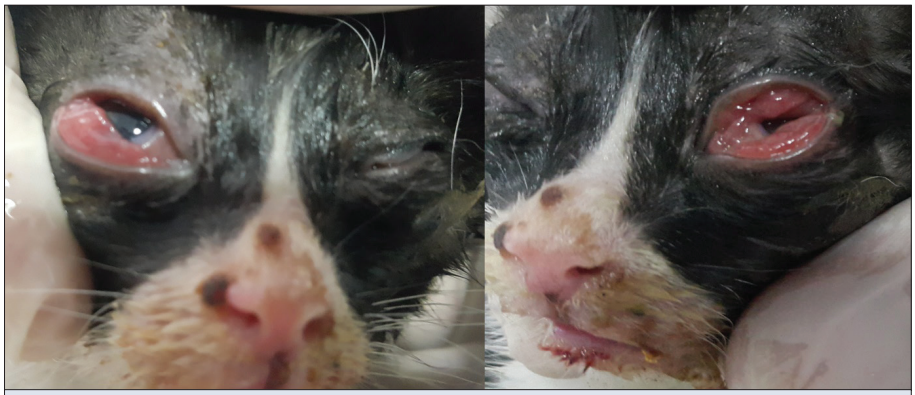

Fig 5. Bilateral blepharitis, serous eye discharge, severe conjunctival swelling (edema, chemosis), nictitating membrane protrusion and bilateral nasal discharge, in an FHV high-positive kitten

only 2 cats. With FCV S4 strong positive result, moderate gingivitis was detected in 5 eyes lesioned kittens, 4 of which were siblings.

Discussion

FHV and FCV are among the most common causes of both upper respiratory tract infections and eye diseases in cats, and FPLV is less effective [9,10]. Particularly, agents transmitted during the intrauterine or postnatal period cause neonatal eye diseases in kittens [3]. In recent years, many epidemiological and prevalence studies have been carried out on these ocular disease agents [3,11-16]. In this study, we aimed to evaluate the serology and clinical findings of FHV, FCV and FPLV together and to interpret how the results to be obtained can be used in the evaluation of clinical cases.

Although the Calicivirus is a well-known viral agent that causes upper respiratory tract infection, gingivostomatitis,

and lameness in cats all over the world, it is also an important ocular disease agent in this species [5,17]. In this study, FCV S3 and above positive titer results were determined in kittens with eye lesions, except for only 1 kitten, in which FCV S2 was weakly positive and FHV S5 strongly positive results were detected. Besides, FCV S4 strongly positive, FHV and FPVL S0 negative titer results were determined in 5 of 20 kittens, while FHV and FPVL S2 weakly positive or below negative titer results were determined in 7 kittens. However, S4's strong positive and above result of FHV in 2 kittens and S3 strong positive result and above FPLV in 4 kittens were determined. As also stated in studies [5,17] above, FCV plays an active and dominant role as a viral eye disease agent, despite local differences.

Kittens receive varying levels of maternal antibodies via colostrum in the first hours after birth, and the amount of antibodies consumed with colostrum varies with the number of kittens, frequency of lactation, and antibody titer contained [18]. Besides, individual antibody levels can vary between siblings, and while some kittens will show no clinical signs and infection, others may develop an infection, which may even be fatal. High positive titers in kittens with clinical signs may indicate an active response, while low positive titers from passively acquired antibodies may reflect maternal antibodies and decrease over time [19]. Most of the kittens included in the study were street-owned or strayed kittens, so the level of maternal antibodies is unknown. It is thought that in kittens without clinical signs, the lack of vaccination, and the high positive titers may be due to the individual immune difference in the maternal antibody or another active asymptomatic infection. Instead, low positive or negative antibody titers in kittens with and without clinical signs may be associated with decreasing maternal antibody levels over time or not being exposed to the viral agent.

The authors state that other disease factors may play a role as cofactors in the changing clinical presentation [5,20]. Conjunctivitis (edema and hyperemia), corneal opacity, iris staphyloma, panophthalmitis, symblepharon, keratitis, corneal edema and microphthalmia were most common in the study *(Fig. 2)*. Except for two kittens with only serous discharge, most kittens had mucopurulent discharge and FCV was detected in each kitten. The determination of S3-S4 as the most detected value in the FCV titer shows that the calicivirus is dominant in the clinical appearance. In this study, in addition to the common symptoms seen in 4 sibling cats, changes in the severity of the clinical appearance, both eye and systemic, were determined. Likewise, in terms of systemic effects, nasal discharge and mouth lesions also differ between 4 siblings. While S2 low positive titers of FHV and FPLV were detected in one of the sibling kittens, FVH S0 and FPLV S1-2 and 3 titers were detected in the other 3 sibling kittens. Considering the titers in these patients, it was thought that variable titer co-factor viruses might be effective in varying clinical manifestations.

In this study, the antibody titers of FCV, FHV and FPLV in neonatal cats were determined with the Dot-ELISA kit and the clinical appearance and prevalence of eye lesions were evaluated. According to reports, FHV is the most prevalent etiology of ocular lesions in kittens [3,20-22]. In the presented study, however, the most common antibodies against FCV were detected. As stated before other factors may have effects on the clinical appearance of feline viral ocular diseases [5,20]. To determine and eliminate these factors a more comprehensive study needs to be performed on this subject. Although the Dot-ELISA test kit data gave faster results in detecting the disease compared to other test methods, i.e. Virus Neutralization or Hemagglutination Inhibition [7] and confirmed the clinical appearance, Our findings show that the calicivirus may cause neonatal ophthalmia more commonly than the herpes virus, and clinical examination findings should be more important and prioritized for clinicians in the evaluation and treatment of neonatal ophthalmic patients.

Availability of Data and Materials

The authors declare that data supporting the study findings are also available to the corresponding author (Ç. Gültekin).

Financial Support

This research did not receive any financial support.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Statement

The use of the data constituting the study was approved by the Local Ethics Committee (Approval no: 2021/138).

Author Contributions

ÇG: conceptualization, methodology, investigation, writing-original draft. SS: conceptualization, methodology, writing-review and editing. FEÖ: methodology, supervision, writing-review and editing.

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