# An Eye on COVID-19: A Meta-analysis of Positive Conjunctival Reverse Transcriptase–Polymerase Chain Reaction and SARS-CoV-2 Conjunctivitis Prevalence

Nicole M. Sopp, OD, FAAO[1]* and Vandhana Sharda, OD, MS, FAAO[1]

SIGNIFICANCE: This analysis and review demonstrate that, although emerging data indicate that the prevalence of severe acute respiratory coronavirus 2 (SARS-CoV-2) on the ocular surface and coronavirus disease 2019 (COVID-19) conjunctivitis is rare, the ocular surface remains of interest as a potential inoculation and transmission site for SARS-CoV-2. Continued safety precautions should be taken as more data become available.

COVID-19, caused by SARS-CoV-2, is a novel, global pandemic that has infected millions and, up to this point, caused more than two million fatalities worldwide. The ocular surface has become of interest as a possible vector for transmission by acting as a direct inoculation site, being a conduit for the virus into the respiratory system or as a method of transmission from potentially infected conjunctiva or tears. The components necessary for SARS-CoV-2 to theoretically infect ocular tissues are present: binding receptors (angiotensin-converting enzyme 2 and cluster of differentiation 147) and mechanisms for cell entry (transmembrane protease serine 2 and cathepsin L). This meta-analysis of COVID-19 prevalence data indicates that SARS-CoV-2 RNA has been infrequently found in conjunctival samples when tested with reverse transcriptase–polymerase chain reaction. This review estimates the prevalence of SARS-CoV-2 on the ocular surface and prevalence of conjunctivitis in patients with laboratory-confirmed COVID-19. There is much to be learned regarding ocular tropism of SARS-CoV-2.
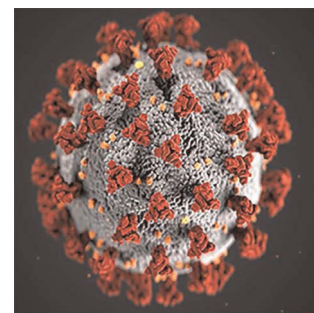
Author Affiliations:
[1]Atrius Health, Newton, Massachusetts
*nicoleuga@hotmail.com

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory coronavirus 2 (SARS-CoV-2), is currently a global pandemic that has, up to the point this article was submitted, infected more than 94 million people worldwide and has been attributed to more than 2 million fatalities according to Worldometer. The novel virus was first identified in the province of Wuhan in China in December 2019. Coronaviruses belong to Coronaviridae, a family of enveloped, positive-sense, single-stranded RNA viruses, and are zoonotic, infecting birds and mammals. COVID-19 is presumed to spread by respiratory droplets and contact through virus-infected fomites, although other transmission routes such as aerosols and fecal-oral are being further studied. Like severe acute respiratory syndrome coronavirus and Middle Eastern respiratory syndrome coronavirus, SARS-CoV-2 is known to cause lower respiratory tract infection, respiratory distress syndrome, and pneumonia. Although originally believed to be primarily a respiratory disease, recent data suggest that COVID-19 results in a prothrombotic state, leading to arterial and venous thrombosis.[1]

The role of the eye and ocular system in COVID-19 is currently under intensive study as a potential transmission site on account of the natural exposure to potential airborne droplets and aerosols, as well as the anatomical relationship to the respiratory tract. The ocular surface may act as a direct inoculation site, a conduit for the virus into the respiratory system or as a method of transmission from potentially infected conjunctiva or tears. There are a number of reports of cases of conjunctivitis in COVID-19–positive patients, and the current prevalence is investigated in this article.[2–6] Further understanding of the ocular transmission of COVID-19 can guide eye care providers in enhancing in-office safety for themselves and patients, as well as in mitigating disease spread to the general public.

GENOMICS/MORPHOLOGY

Coronaviruses, named for the crown-like spike proteins on the viral surface seen on microscopy, are enveloped, positive-sense, single-stranded RNA viruses with a 5′-terminal cap structure and poly(a) sequence at the 3′, ranging in size from 26 to 32 kb, making coronavirus the largest genome of known RNA viruses. Phylogenetically, coronaviruses belong to the family Coronaviridae, order Nidovirales, and are further categorized into four genera: α-coronavirus, β-coronavirus, γ-coronavirus, and δ-coronavirus. Currently, only α-coronaviruses and β-coronaviruses are known to cause human disease.[7] Severe acute respiratory syndrome coronavirus, Middle Eastern respiratory syndrome coronavirus, and SARS-CoV-2 are all classified within the β-coronavirus genera.

All human coronaviruses are zoonotic in origin. The novel coronavirus is likely to have originated in bats, which serve as a natural reservoir for the virus.[8] SARS-CoV-2, like other β-coronaviruses, enters the host cell by binding to a host receptor via viral spike protein. Angiotensin-converting enzyme 2 and, more recently, cluster of differentiation 147 have been identified as important human receptors for coronavirus along with transmembrane protease serine 2, which is necessary for protein priming and entry.[9,10] After interaction with angiotensin-converting enzyme 2 or cluster of differentiation 147,

the virus is endocytosed and cathepsin L must further cleave the virus before it can fuse with the endosome membrane and release RNA into the cytosol.[11] The glycoprotein of SARS-CoV-2 has been determined to bind to human angiotensin-converting enzyme 2 receptors with a 10- to 20-fold higher affinity than severe acute respiratory syndrome coronavirus 1.[12]

A critical binding receptor of SARS-CoV-2, angiotensin-converting enzyme 2, the first known human homolog of angiotensin-converting enzyme, was discovered in 2000 to be a negative regulator of the renin-angiotensin system that counterbalances angiotensin-converting enzyme activity by catalyzing the conversion of angiotensin II to the vasodilatory and antihypertrophic peptide angiotensin 1 to 7 and has been determined to have a protective role in cardiovascular disease.[13–15] Cluster of differentiation 147, otherwise known as basigin or EMMPRIN, is a type 1 transmembrane protein belonging to the immunoglobulin superfamily.[11] These key proteins, along with transmembrane protease serine 2 and cathepsin L, have been a focus of investigation for how COVID-19 infects humans and treatment development.

VIRAL LOAD/VIRAL SHEDDING

A recent comprehensive meta-analysis has found the median time of SARS-CoV-2 clearance from upper and lower respiratory tract samples to be 3.5 and 6 days, respectively. The analysis found no detectable live virus culture beyond 9 days of symptoms despite high viral RNA loads. SARS-CoV-2 viral load seems to peak in the upper respiratory tract within the first week of illness, making affected patients most infectious in the first week of illness. This review goes on to report that several studies have demonstrated that viral load peaks during the prodromal phase of this illness, with most studies showing faster viral clearance in asymptomatic individuals. However, as the data are limited for individuals who are asymptomatic, further study is needed to understand the role of transmission and degree of live virus shedding in these asymptomatic individuals.[16]

CURRENT OCULAR IMPLICATIONS

COVID-19 is primarily known as a respiratory illness, but ocular, gastrointestinal, and prothrombotic complications have also been documented.[1,5,17,18] The ocular surface has been of particular interest in enhancing understanding of viral transmission because of the eyes' natural exposure to droplets and aerosols, as well as easy inoculation potential via direct or indirect touching of the eye and face. The connection to the respiratory system via the nasolacrimal drainage pathway also accentuates the ocular tissue's potential role in serving as a conduit for respiratory infectivity. It has also theorized that COVID-19 can lead to hematogenous infection of the lacrimal gland; however, this requires further study.[19]

A focus of the ocular surface is whether it is an environment that could facilitate host cell entry. Angiotensin-converting enzyme 2 and transmembrane protease serine 2 have been found in both the cornea and conjunctiva, although studies have found conflicting degrees of expression of angiotensin-converting enzyme 2 within the conjunctival samples.[13,20,21] Cathepsin L and cluster of differentiation 147 have also been isolated in the cornea and conjunctiva in moderate to high expression.[11] Transmembrane protease serine 2 has been found to exist in the same corneal cells as angiotensin-converting enzyme 2, although reported cases of COVID-19 keratitis have been very limited at this point, which may be due to tear dynamics and its antimicrobial compounds.[22] More research is necessary to validate these results as heterogeneity, or differences between other conjunctival cells not sampled, as well as different receptors involved, cannot be ruled out.

The eyes and ancillary tissues serving as a conduit for viral transmission to the respiratory system continue to be of concern. In a pivotal study by Deng et al.,[23] two rhesus monkeys were inoculated with SARS-CoV-2 via the conjunctiva, whereas another rhesus was inoculated via the intratracheal route and other via the intragastric route. Investigation revealed mild interstitial pneumonia confirmed via radiography and post-mortem immunohistochemistry in the macaques inoculated via conjunctiva. The researchers were able to detect viral load in nasal and throat swabs from 1 to 7 days after conjunctival inoculation. Viral load was also present in conjunctival swabs of monkeys inoculated only via conjunctiva and for solely 1 day post-inoculation before becoming undetectable, demonstrating virus migration from the conjunctiva to the respiratory tract.[23]

At the time this article was written, the prevalence of conjunctivitis in COVID-19 remains controversial, with data ranging from 0.9 to 36%.[5,24] Although no cases of severe acute respiratory syndrome coronavirus–confirmed conjunctivitis could be found with literature review, severe acute respiratory syndrome coronavirus RNA has been isolated in tears.[25] This is paradoxical because the other β-coronaviruses are known to also bind to host cells via S-protein and angiotensin-converting enzyme 2 receptor, pointing to the possibility of greater infectivity of SARS-CoV-2. Hui et al.[26] isolated SARS-CoV-2 from nasopharyngeal aspirate and throat swab of a patient with confirmed COVID-19 and demonstrated conjunctival tropism using *ex vivo* cultures of human conjunctiva with controls, as well as compared it with tropism of severe acute respiratory syndrome coronavirus. Immunohistochemical staining showed the *ex vivo* conjunctiva infection and viral replication, but more extensively by SARS-CoV-2 than severe acute respiratory syndrome coronavirus, supporting the conjunctiva as an inoculation site.[26]

Signs of COVID-19 conjunctivitis have included mild hyperemia, follicular reaction of the palpebral conjunctiva, chemosis, epiphora, watery discharge, and eyelid edema.[27] COVID-19–associated conjunctivitis has been documented to be both unilateral and bilateral. A cross-sectional study by Güemes-Villahoz et al.[28] found that 54% of cases were unilateral, a striking comparison with adenoviral conjunctivitis, which has a propensity to be sequentially bilateral. Curiously, the study by Güemes-Villahoz et al.[28] did not document any petechiae or subconjunctival hemorrhages in their 35 conjunctivitis cases, which is interesting on account of the vascular or thrombotic complications reported with the virus. No membranes or pseudomembranes have been documented in the literature up to this point, but there is one case of a nasopharyngeal reverse transcriptase–polymerase chain reaction COVID-19–positive patient who developed keratoconjunctivitis.[28,29]

ANALYSES OF CONJUNCTIVAL VIRAL RNA/CONJUNCTIVITIS

A full literature search using the PubMed database identified seven clinical studies to include in this review. Although many samples have been collected, SARS-CoV-2 RNA has been infrequently isolated in conjunctivae and tears using reverse transcriptase–polymerase

chain reaction, and up to this point, live virus has not been isolated via conjunctival cell culture. Conjunctival swabs were analyzed with reverse transcriptase–polymerase chain reaction of 67 patients, with 63 of those patients having laboratory-confirmed SARS-CoV-2 pneumonia and 4 having suspected SARS-CoV-2 pneumonia. Only one patient had a positive conjunctival reverse transcriptase–polymerase chain reaction result, and no patients had ocular symptoms.[30] Xia et al.[2] studied conjunctival and tear swabs of 30 laboratory-confirmed COVID-19 patients, 21 with common type COVID-19 and 9 with severe type COVID-19. They found only one positive conjunctival reverse transcriptase–polymerase chain reaction in one common-type patient who also had the study's only case of conjunctivitis. All other patient conjunctival swabs were negative. Xie et al.[31] swabbed the ocular surface of 33 patients without any ocular manifestations and found 2 strong positives on conjunctival swabs taken 7 days after diagnosis of COVID-19 by analysis of nasopharyngeal swabs with reverse transcriptase–polymerase chain reaction. Of a total of 121 patients managed in the Rennin Hospital of Wuhan University who received conjunctival swabs, only 3 (2.5%) were positive for SARS-CoV-2 detection.[3] In a cross-sectional study by Zhang et al.,[4] only two patients with conjunctivitis with laboratory-confirmed COVID-19 were identified. Of the two conjunctivitis patients, only one was found to have SARS-CoV-2 RNA fragments on reverse transcriptase–polymerase chain reaction analysis of ocular swab, indicating low likelihood but potential of infection through the ocular surface.[4] A retrospective study out of a hospital center in Hubei Province, China, found that, of 28 laboratory-confirmed COVID-19 cases, 2 patients (5.2%) also yielded reverse transcriptase–polymerase chain reaction–positive conjunctival swabs.[5] Hong et al.[6] assessed the medical records of 56 laboratory-positive COVID-19 patients and found that 2 had developed conjunctivitis and only 1 had a positive conjunctival reverse transcriptase–polymerase chain reaction.

The prevalence of positive conjunctival reverse transcriptase–polymerase chain reaction outcomes and conjunctivitis diagnoses in the total study sample and in the cohort of only laboratory-confirmed COVID-19 patients is modeled in Table 1. Estimated prevalence of conjunctival reverse transcriptase–polymerase chain reaction results positive for SARS-CoV-2 RNA in patients with respiratory laboratory-confirmed COVID-19 is provided in Table 2. A plot of the data from Table 2 is provided in Fig. 1. Table 3 displays estimated prevalence of conjunctivitis in patients with COVID-19 confirmed by respiratory or blood sample reverse transcriptase–polymerase chain reaction. The data from Table 3 are plotted in Fig. 2.

CASE REPORTS

There have been other anecdotal case reports of SARS-CoV-2–related ocular findings. Hu et al.[32] found SARS-CoV-2 RNA via reverse transcriptase–polymerase chain reaction and next-generation sequencing in the ocular swab of a COVID-19 patient with nasolacrimal duct obstruction 2 weeks after the nasopharyngeal swab became negative. Navel et al.[33] documented a case of bilateral pseudomembranous conjunctivitis in a hospitalized COVID-19 patient. However, the literature did not mention if the COVID-19 diagnosis was laboratory-confirmed and conjunctival swabs were negative. Salducci and La Torre[34] reported a case of severe viral conjunctivitis in a respiratory reverse transcriptase–polymerase chain reaction–positive patient on the Princess Diamond Cruise Ship with no conjunctival swab performed. Daruich et al.[35] reported a patient with unilateral conjunctivitis diagnosed via telemedicine evaluation who developed a severe headache and fever within 3 hours and then cough and severe dyspnea within 12 hours. The patient's nasopharyngeal swab was positive for SARS-CoV-2

TABLE 1. Meta-analysis of positive conjunctival RT-PCR results and conjunctivitis in patient with COVID-19

	N = total patient sample	N$_1$ patients with laboratory-confirmed COVID-19*	No. positive conjunctival RT-PCR in total patient sample	% of Total patient sample with positive conjunctival RT-PCR (%)	% of Lab-confirmed COVID-19 with positive conjunctival RT-PCR (%)
Zhou et al.[30]	67	63	1	1.5	1.6
Xia et al.[2]	30	30	1	3.3	3.3
Xie et al.[31]	33	33	2	6.1	6.1
Zhou et al.[3]	121	121	3	2.5	2.5
Zhang et al.[4]	102	72	2	2.0	2.8
Wu et al.[5]	38	28	2	5.3	7.1
Hong et al.[6]	56	56	1	1.8	1.8

	No. conjunctivitis cases in total patient sample	% of Total patient sample with conjunctivitis (%)	% of Lab-confirmed COVID-19 with conjunctivitis (%)	No. positive conjunctival RT-PCR with conjunctivitis
Zhou et al.[30]	0	0	0	0
Xia et al.[2]	1	3.3	3.3	1
Xie et al.[31]	0	0	0	0
Zhou et al.[3]	8	6.6	6.6	1
Zhang et al.[4]	2	2.0	2.8	1
Wu et al.[5]	12	31.6	42.9	2
Hong et al.[6]	2	3.6	3.6	1

*Laboratory-confirmed COVID-19 with RT-PCR technique of respiratory or blood sample. COVID-19 = coronavirus disease 2019; RT-PCR = reverse transcriptase–polymerase chain reaction.

TABLE 2. Estimation of prevalence of viral RNA in conjunctiva in laboratory-confirmed COVID-19 patients

Studies	Estimated prevalence	95% CI	(+)Conj. RT-PCR/total lab-confirmed COVID-19*
Zhou et al.[30]	0.016	(0.000–0.085)	1/63
Xia et al.[2]	0.033	(0.001–0.172)	1/30
Xie et al.[31]	0.061	(0.007–0.202)	2/33
Zhou et al.[3]	0.025	(0.005–0.071)	3/121
Zhang et al.[4]	0.028	(0.003–0.097)	2/72
Wu et al.[5]	0.071	(0.009–0.235)	2/28
Hong et al.[6]	0.018	(0.000–0.096)	1/56
Overall	0.030	(0.016–0.051)	12/403

*Number of positive conjunctival RT-PCR/number of COVID-19 patients laboratory confirmed with RT-PCR of respiratory or blood samples. CI = confidence interval; Conj. = conjunctivitis; COVID-19 = coronavirus disease 2019; RT-PCR = reverse transcriptase–polymerase chain reaction.

RNA, demonstrating that conjunctivitis could be the first sign of respiratory distress.[35] Colavita et al.[36] reported on a patient who traveled from Wuhan, China, to Italy whose sputum was reverse transcriptase–polymerase chain reaction positive for SARS-CoV-2. An ocular swab was taken 3 days after hospital admission because of persistent conjunctivitis, and there was positive SARS-CoV-2 detection up to day 21 of illness with daily decline of concentration. Viral replication was also confirmed by real-time reverse transcriptase–polymerase chain reaction on RNA purified from spent cell growth medium, indicating ocular secretions may contain infectious virus.[36] Chien et al.[37] reported a 30-year-old patient with confirmed COVID-19 who developed bilateral SARS-CoV-2 follicular conjunctivitis confirmed with reverse transcriptase–polymerase chain reaction on day 13 of illness. Conjunctival reverse transcriptase–polymerase chain reaction remained positive until day 19. Scalinci and Battagliola[38] reported five cases of conjunctivitis that were followed by laboratory-confirmed COVID-19 with none ever experiencing fever, malaise, or respiratory symptoms, concluding that conjunctivitis was the only presenting sign of COVID-19. Cheema et al.[29] reported a case of keratoconjunctivitis in a 29-year-old patient with no other comorbidities. The patient developed ocular hyperemia in one eye, along with rhinorrhea, cough, and nasal congestion. Upon evaluation, the patient had subepithelial infiltrates with overlying epithelial defects along with a possible pseudodendrite. A nasopharyngeal swab 5 days later was positive for SARS-CoV-2 through reverse transcriptase–polymerase chain reaction, and retrospective testing of the patient's bacterial conjunctival swab revealed a weakly positive reverse transcriptase–polymerase chain reaction result (37 cycles for the conjunctival swab versus 23 to 25 cycles for the nasopharyngeal swab).[29] The case reports described are summarized in Table 4.

POTENTIAL FOR POSTERIOR OCULAR DISEASE

Up to this point, human coronaviruses have not been found to be the cause of ocular disease beyond potentially conjunctivitis. However, the possibility of the implication of coronaviruses in more posterior, sight-threatening pathology cannot be ignored. Marinho et al.[39] analyzed 12 symptomatic patients. Four patients were found to have subtle cotton wool spots and microhemorrhages along the vascular arcades, representing the first possible documentation of COVID-19–associated retinal findings in humans, although it is unclear if there were any comorbidities that would
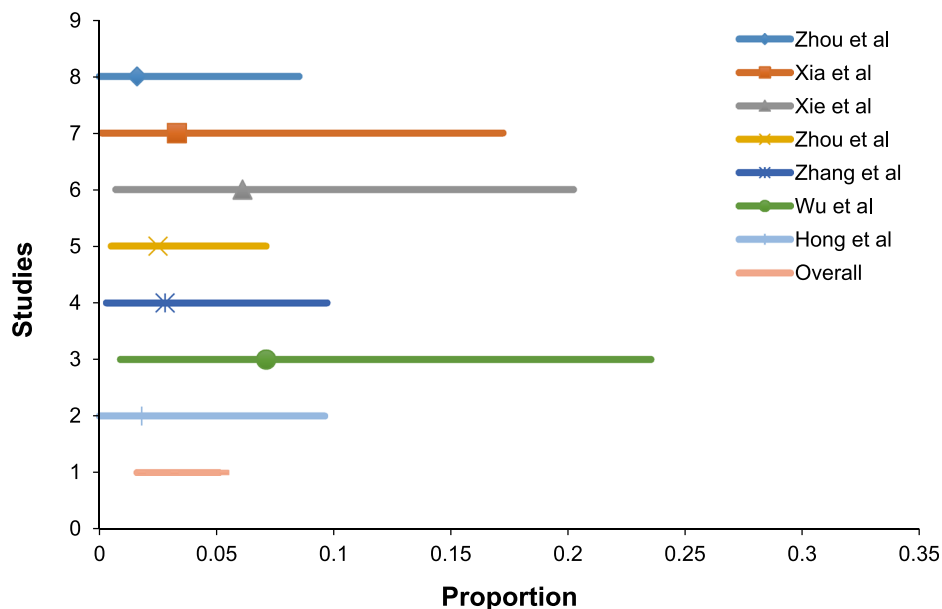

FIGURE 1. Plot of prevalence of positive conjunctival RT-PCR in laboratory-confirmed COVID-19 patients. COVID-19 = coronavirus disease 2019; RT-PCR = reverse transcriptase–polymerase chain reaction.

TABLE 3. Estimation of prevalence of conjunctivitis in laboratory-confirmed COVID-19 patients

Studies	Estimated prevalence	95% CI	No. conjunctivitis cases/total lab-confirmed COVID-19*
Zhou et al.[30]	0.000	(0.000–0.057)	0/63
Xia et al.[2]	0.033	(0.001–0.172)	1/30
Xie et al.[31]	0.000	(0.000–0.106)	0/33
Zhou et al.[3]	0.067	(0.029–0.126)	8/121
Zhang et al.[4]	0.028	(0.003–0.097)	2/72
Wu et al.[5]	0.429	(0.245–0.628)	12/28
Hong et al.[6]	0.036	(0.004–0.123)	2/56
Overall	0.062	(0.041–0.090)	25/403

*Number of cases of conjunctivitis/number of COVID-19 patients laboratory confirmed with RT-PCR of respiratory or blood samples. CI = confidence interval; COVID-19 = coronavirus disease 2019; RT-PCR = reverse transcriptase–polymerase chain reaction.

have confounded the findings.[39] Pirraglia et al.[40] studied 43 patients with severe COVID-19 and found no retinal manifestations with exception of 1 patient with an opportunistic unilateral posterior chorioretinitis whose aqueous tap was SARS-CoV-2 negative. Another publication by Invernizzi et al.[41] found that the diameter of retinal arteries and veins were larger in COVID-19 subjects than unexposed subjects, and the retinal vein diameter directly correlated with COVID-19 disease severity using funduscopy 30 days after symptom onset. It is unclear whether the vascular response is secondary to the virus itself or immune response of the host.[41]
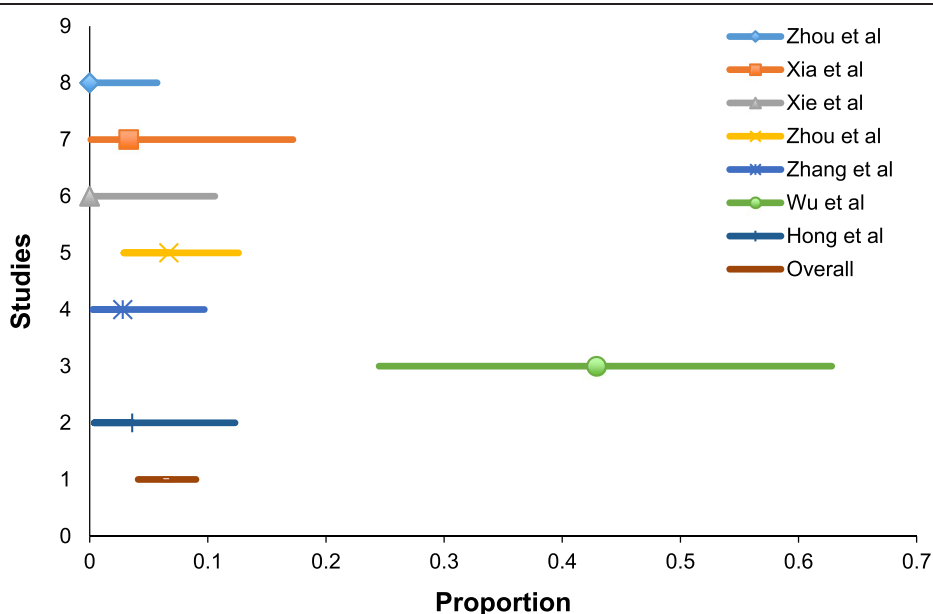
The feline coronavirus (an α-coronavirus), which infects wild and domestic cats, is classified into two biotypes: Feline enteric coronavirus and feline infectious peritonitis virus. Feline enteric coronavirus is mostly benign, affecting the majority of the feline population, whereas feline infectious peritonitis virus has poor prognosis. Feline infectious peritonitis virus results in widespread vasculitis and inflammation of various ocular segments with ocular manifestations including granulomatous anterior uveitis and choroiditis, with retinal detachment and retinal vasculitis.[19,39] The murine coronavirus mouse hepatitis virus is divided into two biotypes, with one mainly affecting the gastrointestinal tract and the other containing strains that affect various organ systems including the central nervous system and the hepatic and pulmonary systems. Strains of mouse hepatitis virus have been found to infect glial cells, astrocytes, oligodendrocytes, and microglia. Posterior ocular infection has led to the development of murine models for study of retinal degeneration or experimental coronavirus retinopathy used to examine genetic and host immune responses that can lead to retinal disease. Another strain has been used to create models of virus-induced optic neuritis for study of the theory of a viral etiology of multiple sclerosis.[19,42–44]

DISCUSSION

Up to this point, conjunctivitis can be considered an uncommon manifestation of COVID-19 with a prevalence estimation of 6.2% (95% confidence interval, 4.1 to 9.0%) using binomial calculation from the review of literature conducted with PubMed search (Table 1, Table 3, Fig. 2). This estimation was calculated considering only COVID-19 cases confirmed by either respiratory or blood specimen reverse transcriptase–polymerase chain reaction. This analysis does have its limitations. The studies included were homogenous in terms of testing techniques, although most did not indicate total cycle threshold to determine positive reverse transcriptase–polymerase chain reaction, and Xia et al.[2] and

FIGURE 2. Plot of prevalence of conjunctivitis diagnoses in laboratory-confirmed COVID-19 patients. COVID-19 = coronavirus disease 2019.

TABLE 4. Ocular COVID-19 case reports summarized

Hu et al.[32]	(+)Conjunctival RT-PCR in eye with nasolacrimal duct obstruction
Navel et al.[33]	Case of bilateral pseudomembranous conjunctivitis in COVID-19 patient unclear whether COVID-19 laboratory confirmed; conjunctival RT-PCR negative
Salducci and La Torre[34]	Severe conjunctivitis in laboratory-confirmed COVID-19 patient on Princess Diamond Cruise Ship; conjunctival swab not performed
Daruich et al.[35]	Unilateral conjunctivitis diagnosed via telemedicine evaluation and development of headache within 3 h and cough/severe dyspnea within 12 h; laboratory-confirmed COVID-19
Colavita et al.[36]	Persistent conjunctivitis with (+)conjunctival RT-PCR in laboratory-confirmed COVID-19 patient; viral replication confirmed by real-time RT-PCR on RNA purified from spent cell growth medium
Chien et al.[37]	(+)Conjunctival RT-PCR with bilateral conjunctivitis on day 13 of illness with RT-PCR remaining (+) until day 19
Scalinci and Battagliola[38]	Five cases of conjunctivitis in laboratory-confirmed COVID-19 patients who never developed fever, malaise, or respiratory problems; no conjunctival swab performed
Cheema et al.[29]	Unilateral keratoconjunctivitis with faint conjunctival RT-PCR positive in nasopharyngeal RT-PCR (+) patient.

COVID-19 = coronavirus disease 2019; RT-PCR = reverse transcriptase–polymerase chain reaction.

Zhou et al.[30] reported a difference of 40 cycles and 45 cycles, respectively. The variance in threshold in determining positivity of infection may have skewed the data results in either a positive or negative direction. There may be publication bias because studies included were only from a systematic review of PubMed literature. The authors assume that infectivity was from the same strain of COVID-19 virus on account of the time frame and geography. Misdiagnosis of an opportunistic conjunctival infection is a possible confounder that could lead to an overestimation of COVID-19 conjunctivitis prevalence. Only laboratory-positive COVID-19 diagnoses were used to estimate prevalence, but including all suspected or solely clinically diagnosed cases would have driven the prevalence down further. Greater sample sizes need to be studied to validate this conclusion. Although this analysis did not stratify prevalence of conjunctivitis within levels of COVID-19 disease severity, a meta-analysis by Lofreddo et al.[45] did find that the rate of conjunctivitis was 3 and 0.7% in severe and nonsevere cases, respectively.

Albeit conjunctivitis is not a common manifestation, clinicians should continue to obtain thorough review of systems and consider noncontact screening for fever. Proper education on frequent handwashing to prevent transmission should still be provided following the pattern of any viral conjunctivitis. Clinicians should be aware of the uncommon but benign conjunctivitis that has been associated with COVID-19 to provide concerned patients palliative recommendations including cool compresses and artificial tears using telemedical strategies.

The report by Scalinci and Battagliola[38] of the cases of conjunctivitis as the presenting and sole symptom of COVID-19 is concerning but anecdotal at this time. No conjunctival cell analysis was performed, and it is possible that conjunctivitis was secondary to opportunistic infection or general immune response from the respiratory infection. Studies with larger population sizes providing conjunctival and nasopharyngeal swab reverse transcriptase–polymerase chain reaction assessment for cases of viral conjunctivitis would be helpful to capture a more accurate understanding of COVID-19 conjunctivitis prevalence and its relationship to respiratory illness. Hopefully, as with the previous SARS outbreak, the COVID-19 infection rate will be significantly reduced before sufficient testing can be achieved.

The detection of SARS-CoV-2 RNA in the tears and conjunctiva should heighten suspicion of the eyes as a possible transmission site of the virus. However, detection of SARS-CoV-2 in ocular samples also remains uncommon at an estimated 2.7% (95% confidence interval, 1.4 to 4.8%) using binomial calculation with review of literature (Table 1, Table 2, Fig. 1). Again, only laboratory-confirmed diagnoses were used in calculation, so the prevalence is likely overestimated. The rarity of positive ocular testing may simply be too low of a viral load for reverse transcriptase–polymerase chain reaction detection or patients are being sampled at an inopportune time in the course of their disease. False negatives may also occur because of mutations of the SARS-CoV-2 genome or poor collection, transportation, or handling.

Another consideration is the expression of SARS-CoV-2 receptors on the ocular surface. This should continue to raise questions as to whether, like feline and murine coronaviruses, SARS-CoV-2 can enter the body through the eyes, but, at this time, there is no evidence to support this theory. The low expression of both angiotensin-converting enzyme 2 and transmembrane protease serine 2 found in prior studies may also be the justification for the limited prevalence of COVID-19 ocular pathology and discredit the eye as a direct inoculation site.[13,20] The demonstration of SARS-CoV-2 tropism in donor conjunctiva by Hui et al.[26] does support possible conjunctival infection; however, the eye's excellent defenses of a turbulent tear layer, antimicrobial proteins, and immunoglobulins may not make this likely in an *in vivo* scenario.

To the best of the authors' knowledge, there has not been a positive reverse transcriptase–polymerase chain reaction conjunctival swab without a positive nasopharyngeal swab. This suggests that rather than the ocular surface serving as a direct inoculation site for SARS-CoV-2, the nasolacrimal duct may act as a conduit for the virus into the respiratory system. The nasolacrimal transmission pathway is also supported by the rhesus macaque study by Deng et al.[23] in which direct conjunctival inoculation led to nasopharyngeal SARS-CoV-2 RNA detection and development of mild interstitial pneumonia. There was viral RNA detection in the conjunctiva only 1 day post-inoculation and in the throat 1 to 7 days post-inoculation. It is unclear whether the $1 \times 10,651$ $TCID_{50}$ of SARS-CoV-2 inoculation dose could be authenticated in real-world environment.[23]

These analysis and review were conducted based on data from experimental studies on an adult cohort performed in Asia. These are the data that were available at the time of this research on account of COVID-19 first spreading through China. It will be interesting to see if the prevalence of SARS-CoV-2 on the ocular surface and prevalence of COVID-19 conjunctivitis remain comparable with the data gathered in the western hemisphere. Scientific evidence of the role of the conjunctiva in COVID-19 is rapidly evolving. Future review should also control for patient use of ocular drops, contact lenses, or even punctal plugs, as each may have influence

over conjunctival swab outcomes and prevalence values. More research is needed as to whether these factors influence tropism.

The isolation of SARS-CoV-2 RNA from the ocular surface illustrates the importance of frequent handwashing, wearing of disposable gloves, and donning proper personal protective equipment while in close patient contact in a clinical setting to avoid viral transmission. Physical distancing should be observed in patient waiting areas. The emerging evidence of ocular transmission via the nasolacrimal duct accentuated by the aerosol transmission route should caution eye care providers to consider the use of goggles and face shields given the amount of time spent in close patient proximity during eye examinations. Talking should also be limited during slit-lamp examination, and protective slit-lamp screens should be used to minimize risk of droplet transmission. Hand hygiene for the general public must continue to be emphasized. Risk and benefit of ocular examination and therefore patient exposure should be carefully assessed individually for each patient.

Tonometry techniques resulting in aerosolization of particles including air-puff tonometry and pneumotonometry should be avoided; instead, eye care providers should opt for other readily available tonometry instruments such as Goldmann, iCare, or Tono-Pen. Although sodium hypochlorite is a likely disinfectant for prisms, disposable prisms are recommended when performing Goldmann applanation tonometry when possible.[46] Equipment and surfaces should be disinfected with a virucidal agent in between each patient examination.

The presence of SARS-CoV-2 RNA swabbed from conjunctiva and cases of conjunctivitis with laboratory-confirmed COVID-19 cases certainly supports the role of the eye as either a direct inoculation site or conduit for SARS-CoV-2 to the respiratory system. At the time this article was written, live SARS-CoV-2 has not been able to be isolated from human conjunctiva; therefore, true conjunctival infectivity has not been proven. COVID-19 conjunctivitis can be considered as an uncommon manifestation at this time. SARS-CoV-2 RNA has been detected in tears and conjunctiva, although the prevalence is also low. The low prevalence of positive conjunctival reverse transcriptase–polymerase chain reaction in the presence of conjunctivitis may also indicate other modes of ocular inflammation such as ocular

dryness or secondary infection within the hospital setting, further reducing true prevalence measures. More research is needed to understand SARS-CoV-2 to host binding and ocular tropism.

TAKEAWAY POINTS

- Angiotensin-converting enzyme 2 and cluster of differentiation 147, SARS-CoV-2 binding receptors, along with critical proteins transmembrane protease serine 2 and cathepsin L have been isolated in cornea and conjunctiva, although degrees of expression have been variable in research supporting possibility of direct ocular inoculation. Other receptors could also be involved in SARS-CoV-2 infectivity.

- The lacrimal system serving as a conduit to the respiratory system is also a concern. Researchers have detected SARS-CoV-2 RNA in nasal and throat swabs after conjunctival inoculation of rhesus monkeys.[23]

- Conjunctival tropism has been demonstrated using *ex vivo* cultures of human conjunctiva using SARS-CoV-2 isolated from nasopharyngeal aspirate.[26]

- Up to this point, human coronaviruses have not been found to be the cause of ocular disease beyond potentially conjunctivitis. Although research of coronavirus' implication in retinal pathology is ongoing.

- Up to this point, conjunctivitis can be considered an uncommon manifestation of COVID-19 with prevalence estimation of 6.2% (95% confidence interval, 4.1 to 9.0%).

- Detection of SARS-CoV-2 in tears and conjunctival is also uncommon occurring at 2.7% (95% confidence interval, 1.4 to 4.8%); however, this should highlight suspicion of the eye as a possible transmission site of virus and encourage eye care professionals to use appropriate precautions during examinations.

- To the best of the authors' knowledge, there has not been a positive reverse transcriptase–polymerase chain reaction conjunctival swab without a positive nasopharyngeal swab, further supporting the lacrimal system's role as a conduit for the virus to the respiratory system.

REFERENCES

1. Abou-Ismail MY, Diamond A, Kapoor S, et al. The Hypercoagulable State in COVID-19: Incidence, Pathophysiology, and Management. Thromb Res 2020;194:101–15.

2. Xia J, Tong J, Liu M, et al. Evaluation of Coronavirus in Tears and Conjunctival Secretions of Patients with SARS-CoV-2 Infection. J Med Virol 2020;92:589–94.

3. Zhou Y, Duan C, Zeng Y, et al. Ocular Findings and Proportion with Conjunctival SARS-CoV-2 in COVID-19 Patients. Ophthalmology 2020;127:982–3.

4. Zhang X, Chen X, Chen L, et al. The Evidence of SARS-CoV-2 Infection on Ocular Surface. Ocul Surf 2020;18:360–2.

5. Wu P, Duan F, Luo C, et al. Characteristics of Ocular Findings of Patients with Coronavirus Disease 2019 (COVID-19) in Hubei Province, China. JAMA Ophthalmol 2020;138:575–8.

6. Hong N, Yu W, Xia J, et al. Evaluation of Ocular Symptoms and Tropism of SARS-CoV-2 in Patients Confirmed with COVID-19 [published online April 26, 2020]. Acta Ophthalmol 2020. doi:10.1111/aos.14445.

7. Rodriguez-Morales AJ, Bonilla-Aldana DK, Balbin-Ramon GJ, et al. History Is Repeating Itself: Probable Zoonotic Spillover as the Cause of the 2019 Novel Coronavirus Epidemic. Infez Med 2020;28:3–5.

8. Zhou P, Yang XL, Wang XG, et al. A Pneumonia Outbreak Associated with a New Coronavirus of Probable Bat Origin. Nature 2020;579:270–3.

9. Hirano T, Murakami M. COVID-19: A New Virus, but a Familiar Receptor and Cytokine Release Syndrome. Immunity 2020;52:731–3.

10. Perrotta F, Matera MG, Cazzola M, et al. Severe Respiratory SARS-CoV2 Infection: Does ACE2 Receptor Matter? Respir Med 2020;168:105996.

11. Barnett BP, Wahlin K, Krawczyk M, et al. Potential of Ocular Transmission of SARS-CoV-2: A Review. Vision (Basel) 2020;4:40.

12. Wrapp D, Wang N, Corbett K, et al. Cryo-EM Structure of the 2019-nCoV Spike in the Prefusion Conformation. Science 2020;367:1260–3.

13. Ma D, Chen CB, Jhanji V, et al. Expression of SARS-CoV-2 Receptor ACE2 and TMPRSS2 in Human Primary Conjunctival and Pterygium Cell Lines and in Mouse Cornea. Eye (Lond) 2020;34:1212–9.

14. Kuba K, Imai Y, Penninger JM. Multiple Functions of Angiotensin-converting Enzyme 2 and Its Relevance in Cardiovascular Diseases. Circ J 2013;77:301–8.

15. Donoghue M, Hsieh F, Baronas E, et al. A Novel Angiotensin-converting Enzyme-related Carboxypeptidase (ACE2) Converts Angiotensin I to Angiotensin 1–9. Circ Res 2000;87:E1–9.

16. Cevik M, Tate M, Lloyd O, et al. SARS-CoV-2, SARS-CoV, and MERS-CoV Viral Load Dynamics, Duration of Viral Shedding, and Infectiousness: A Systematic Review and Meta-analysis. Lancet Microbe 2021;2:e13–22.

17. Chen L, Liu M, Zhang Z, et al. Ocular Manifestations of a Hospitalised Patient with Confirmed 2019 Novel Coronavirus Disease. Br J Ophthalmol 2020;104:748–51.

18. Gu J, Han B, Wang J. COVID-19: Gastrointestinal Manifestations and Potential Fecal-oral Transmission. Gastroenterology 2020;158:1518–9.

19. Seah I, Agrawal R. Can the Coronavirus Disease 2019 (COVID-19) Affect the Eyes? A Review of Coronaviruses and Ocular Implications in Humans and Animals. Ocul Immunol Inflamm 2020;28:391–5.

20. Lange C, Wolf J, Auw-Haedrich C, et al. Expression of the COVID-19 Receptor ACE2 in the Human Conjunctiva. J Med Virol 2020;92:2081–6.

21. Zhou L, Xu Z, Castiglione GM, et al. ACE2 and TMPRSS2 Are Expressed on the Human Ocular Surface, Suggesting Susceptibility to SARS-CoV-2 Infection. Ocul Surf 2020;18:537–44.

22. Sungnak W, Huang N, Becavin C, et al. SARS-CoV-2 Entry Factors Are Highly Expressed in Nasal Epithelial Cells Together with Innate Immune Genes. Nat Med 2020;26:681–7.

23. Deng W, Bao L, Gao H, et al. Ocular Conjunctival Inoculation of SARS-CoV-2 Can Cause Mild COVID-19 in Rhesus Macaques. Nat Commun 2020;11:4400.

24. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med 2020;382:1708–20.

25. Loon SC, Teoh SC, Oon LL, et al. The Severe Acute Respiratory Syndrome Coronavirus in Tears. Br J Ophthalmol 2004;88:861–3.

26. Hui KPY, Cheung MC, Perera R, et al. Tropism, Replication Competence, and Innate Immune Responses of the Coronavirus SARS-CoV-2 in Human Respiratory Tract and Conjunctiva: An Analysis in *Ex-vivo* and *In-vitro* Cultures. Lancet Respir Med 2020;8:687–95.

27. Napoli PE, Nioi M, d'Aloja E, et al. The Ocular Surface and the Coronavirus Disease 2019: Does a Dual 'Ocular Route' Exist? J Clin Med 2020;9:1269.

28. Güemes-Villahoz N, Burgos-Blasco B, Garcia-Feijoó J, et al. Conjunctivitis in COVID-19 Patients: Frequency and Clinical Presentation. Graefes Arch Clin Exp Ophthalmol 2020;258:2501–7.

29. Cheema M, Aghazadeh H, Nazarali S, et al. Keratoconjunctivitis as the Initial Medical Presentation of the Novel Coronavirus Disease 2019 (COVID-19). Can J Ophthalmol 2020;55(4):e125–9.

30. Zhou Y, Zeng Y, Tong Y, et al. Ophthalmologic Evidence against the Interpersonal Transmission of 2019 Novel Coronavirus through Conjunctiva [published online February 12, 2020]. medRx. doi:10.1101/2020.02.11.20021956.

31. Xie HT, Jiang SY, Xu KK, et al. SARS-CoV-2 in the Ocular Surface of COVID-19 Patients. Eye Vis 2020;7:23.

32. Hu Y, Chen T, Liu M, et al. Positive Detection of SARS-CoV-2 Combined HSV1 and HHV6B Virus Nucleic Acid in Tear and Conjunctival Secretions of a Non-conjunctivitis COVID-19 Patient with Obstruction of Common Lacrimal Duct. Acta Ophthalmol 2020;98:859–63.

33. Navel V, Chiambaretta F, Dutheil F. Haemorrhagic Conjunctivitis with Pseudomembranous Related to SARS-CoV-2. Am J Ophthalmol Case Rep 2020;19:100735.

34. Salducci M, La Torre G. COVID-19 Emergency in the Cruise's Ship: A Case Report of Conjunctivitis. Clin Ter 2020;171:e189–91.

35. Daruich A, Martin D, Bremond-Gignac D. Unilateral Conjunctivitis as First Presentation of Coronavirus Disease 2019 (COVID-19): A Telemedicine Diagnosis. J Fr Ophtalmol 2020;43:e167–8.

36. Colavita F, Lapa D, Carletti F, et al. SARS-CoV-2 Isolation from Ocular Secretions of a Patient with COVID-19 in Italy with Prolonged Viral RNA Detection. Ann Intern Med 2020;173:242–3.

37. Chien L, Liu M, Zhang Z, et al. Ocular Manifestations of a Hospitalized Patient with Confirmed 2019 Novel Coronavirus Diseases. Br J Ophthalmol 2020;104:748–51.

38. Scalinci SZ, Battagliola ET. Conjunctivitis Can Be the Only Presenting Sign and Symptom of COVID-19. IDCases 2020;20:e00774.

39. Marinho P, Marcos A, Romano A, et al. Retinal Findings in Patients with COVID-19. Lancet 2020; 395(10237):1610.

40. Pirraglia M, Ceccarelli G, Cerini A. Retinal Involvement and Ocular Findings in COVID-19 Pneumonia Patients. Sci Rep 2020;10:17419.

41. Invernizzi A, Torre A, Parrulli S, et al. Retinal Findings in Patients with COVID-19: Results from the SERPICO-19 Study. EClinicalMedicine 2020; 27:100550.

42. Doherty MJ. Ocular Manifestations of Feline Infectious Peritonitis. J Am Vet Med Assoc 1971;159: 417–24.

43. Robbins SG, Detrick B, Hooks JJ. Retinopathy following Intravitreal Injections of Mice with MHV Strain JHM. Adv Exp Med Biol 1990;276:519–24.

44. Schindler KS, Kenyon LC, Dutt M, et al. Experimental Optic Neuritis Induced by a Demyelinating Strain of Mouse Hepatitis Virus. J Virol 2008;82: 8882–6.

45. Lofreddo L, Pacella F, Pacella E, et al. Conjunctivitis and COVID-19: A Meta-analysis. J Med Virol 2020;192: 1413–4.

46. Almazyad E, Ameen S, Khan M, et al. Guidelines and Recommendations for Tonometry Use during COVID-19 Era. Middle East Afr J Ophthalmol 2020; 27:73–8.