

# Particulate matter from wildfire smoke in northern Colorado appears to be associated with conjunctivitis in dogs

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## OBJECTIVE

To evaluate ocular surface parameters in dogs with normal eyes when exposed to 3 different air quality index (AQI) categories corresponding to levels of normal air pollutants (“good,” 0 to 50; “moderate,” 51 to 100) and wildfire smoke (“smoke,” 101 to 150).

## ANIMALS

15 privately owned dogs.

## METHODS

A prospective cohort study with dogs living in northern Colorado. Ocular surface parameters (conjunctival chemosis and hyperemia, Schirmer tear test-1, tear film break-up time, fluorescein stain, conjunctival microbiology, etc) were evaluated when the AQI was reported in 1 of the 3 categories (good, moderate, and smoke) for 3 consecutive days. The AQI and air pollutant levels (particulate matter < 2.5 µm in diameter [PM<sub>2.5</sub>], ozone, etc) were retrieved from the AirNow database.

## RESULTS

Due to scheduling conflicts, only 7 dogs were examined during the smoke category. Average AQI in the 3 categories were good, 44.1; moderate, 73.7; and smoke, 103.7. The odds for more severe hyperemia and more severe chemosis for smoke were 5.39 and 7,853.02 times the odds, respectively, when compared to good AQI. Additionally, the odds for more severe chemosis were 34,656.62 times the odds for smoke when compared to moderate AQI. A significant relationship was found between chemosis and PM<sub>2.5</sub>.

## CONCLUSIONS

Exposure to increased AQI related to wildfire smoke caused a significant increase in conjunctivitis. The significant relationship between chemosis and PM<sub>2.5</sub> could indicate that PM<sub>2.5</sub> in wildfire smoke is associated with an inflammatory factor.

## CLINICAL RELEVANCE

Preventive measures (eg, use of eyewash, artificial tears, or eye protection) for dogs that are exposed to wildfire smoke should be instituted to decrease the risk of ocular irritation.

**Keywords:** air quality index, canine, microbiology, wildfire, tear film break-up time

The combination of dry conditions and higher temperatures has led to an increased incidence of wildfires in the western US, southern Europe, and

Australia, with the estimated burned area effectively quadrupling over the past 4 decades.<sup>1-3</sup> Studies<sup>4</sup> in humans have found that wildfire smoke interferes with tear film stability and corresponds to symptoms like itching, burning, and a foreign body sensation in the eye. Additionally, exposure to wildfire smoke has been linked to increased cases of human keratitis, conjunctivitis, and an increase in oxidative stress and cytokine release from human corneal cells.<sup>5,6</sup>

Received May 30, 2024

Accepted July 29, 2024

Published online August 13, 2024

doi.org/10.2460/javma.24.05.0356

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Wildfire smoke contains compounds such as carbon monoxide (CO), polycyclic aromatic hydrocarbons, ozone (O<sub>3</sub>), and particulate matter (PM), many of which have been linked to negative human health side effects.<sup>7,8</sup> The air quality index (AQI) is a score established by the Environmental Protection Agency (EPA) and is measured on a scale from 0 to 500. The AQI score is based on the concentration of each of the five major air pollutants regulated by the US Federal Clean Air Act (CO, O<sub>3</sub>, PM, and nitrogen dioxide [NO<sub>2</sub>]). EPA guidelines<sup>9</sup> specify that an AQI of 0 to 50 is rated “good,” 51 to 100 is “moderate,” 101 to 150 is “unhealthy for sensitive groups,” and 151 to 200 is “unhealthy for the general public.” Our research team demonstrated a significant increase in dogs presenting with infected corneal ulcerations at the Colorado State University’s (CSU) Veterinary Teaching Hospital in 2020 when the state of Colorado was impacted by the 2 largest wildfires in the state’s history.<sup>10</sup> This study illustrated a potential association with poor air quality, smoke, and infected corneal ulcerations in dogs; however, due to the retrospective nature, it was unable to elucidate the underlying mechanism.

The population of humans and domestic pets exposed to wildfire smoke will continue to increase in the future, which necessitates further study into the adverse health effects related to smoke exposure. Investigation into this topic and possible solutions will hopefully lead to decreased morbidity in dogs. The goal of this prospective study was to investigate how AQI categories and wildfire smoke can impact ocular surface parameters in healthy dogs.

## Methods

This study was designed as a prospective cohort study aiming to evaluate 15 healthy dogs at 3 different air quality indexes. Dogs were initially evaluated with a full ophthalmic examination to determine any preexisting ophthalmic diseases before being enrolled in the study. Only dogs with normal ocular parameters and the absence of ophthalmic diseases were included in the study. Dogs belonged to owners who were CSU students or employees and resided in Larimer County, CO (the county for CSU), to ensure they were exposed to similar levels of air quality. Dogs were enrolled in the study with the owner’s written consent, and the study protocol was approved by the CSU IACUC (No. 3539).

## Dogs

Dogs were initially evaluated with a full ophthalmic examination performed by a board-certified veterinary ophthalmologist (MdLH) to assess whether they could be enrolled in the study. The ophthalmic examination included a neuro-ophthalmic examination (menace response, dazzle reflex, pupillary light reflex [direct and indirect], and palpebral reflex), examination of the adnexa and anterior segment with a transilluminator (Welch Allyn), slit-lamp biomicroscopy (KOWA-SL-17), and a fundic examination performed with indirect binocular

ophthalmoscopy using a headset (Keeler Inc) and a 28-diopter condensing lens (Volk) of both eyes.

## Air quality index

The AQI was retrieved from the AirNow database for Larimer County.<sup>9</sup> Once 3 consecutive days with a mean AQI score that fit into the desired air quality category (good, moderate, unhealthy for sensitive groups caused by wildfire smoke [smoke]) were observed, the dogs were presented to the CSU Veterinary Teaching Hospital for an ophthalmic examination. The 3-day average air pollutant levels including fine particulate matter < 2.5 µm in diameter (PM<sub>2.5</sub>), CO, O<sub>3</sub>, and NO<sub>2</sub> were retrieved retroactively using the EPA air quality daily data website.<sup>11</sup> In the case of NO<sub>2</sub>, there were no available data for Larimer County, CO, and the nearest available county data were used from Weld County, CO, which is the closest county to the east. The humidity was also retrieved retroactively using the CSU Fort Collins weather station data website.<sup>12</sup>

## Ophthalmic examination

Ocular surface parameters were evaluated for dogs in the 3 AQI categories (mild, moderate, and smoke). Parameters were evaluated in the same order for every examination. Adnexal and anterior segment was evaluated with slit-lamp biomicroscopy; specifically, chemosis and hyperemia were evaluated and graded on a scale of 0 to 3, consistent with the grading scale established for allergic conjunctivitis by Lourenco-Martin et al.<sup>13</sup> The grading was performed by MdLH who was masked to the patient number and previous score. Photographs of the adnexa and anterior segment were taken of both eyes for documentation using the QuikVue Anterior Segment Imaging Adaptor (VisuScience Meditach Co, Ltd) attached to an iPhone. A Schirmer tear test-1 (STT-1) was performed on both eyes, measured in millimeters per minute (normal reference value > 15 mm/min).<sup>14</sup> Microbiologic culture samples were then collected by placing and gently rotating a culture swab (BBL Culture Swab Plus; Becton, Dickinson and Co) for 5 seconds in the medial canthus/fornix from the right eye and then the left eye (1 pooled sample of both eyes). The swab was submitted to the CSU Veterinary Diagnostic Laboratory for aerobic microbial culture. Fluorescein stain of both eyes was performed following conjunctival sample collection (Fluorescein strip; Bio-Glo; HUB Pharmaceuticals). Tear film break-up time (TFBUT) of both eyes was measured in seconds (normal reference value approx 20 seconds).<sup>15</sup> Tear film break-up time was measured using a 0.5-mL aliquot of eyewash (Bausch & Lomb Inc) combined with a fluorescein strip in a 1-mL syringe, and a drop of the solution was applied to the eye. The eyelids were manually closed and then opened, and the slit-lamp biomicroscopy was set on the cobalt blue filter. The observer (MdLH) counted in seconds until a fluorescein stain break-up was noted as dark spots or lines in the fluorescein stain. This was recorded 3 times per eye, and the mean value for each eye was calculated.<sup>15</sup> Rose Bengal stains of both eyes

(Rose Bengal Ophthalmic Strips; JorVet; Jorgensen Laboratories) were performed following TFBUT. The intraocular pressures (IOPs) were measured in both eyes in millimeters of mercury (TonoVet; normal reference value, 15 to 25 mm Hg).<sup>16</sup>

## Data analysis

Descriptive data are presented as number (n), SD, and range. Residuals were checked for numeric outcomes (STT, IOP, and TFBUT) and found to be approximately normally distributed and homogeneous. Analyses were performed in 2 different ways: each of the 7 variables (categorical AQI, numeric AQI, humidity, PM<sub>2.5</sub>, CO, O<sub>3</sub>, and NO<sub>2</sub>) were used independently to predict each of the ophthalmological outcomes, and then each variable was used together (eliminating any with strong multicollinearity) to predict each outcome. For the outcomes that were numeric (STT, IOP, and TFBUT), linear mixed-effects models were used. For the outcomes that were ordered categories (hyperemia and chemosis), ordinal mixed-effects logistic regression was used. For whether microbiota were isolated in culture (yes or no), binary logistic mixed-effects regression was used. Predictors of categorical AQI, numeric AQI, humidity, PM<sub>2.5</sub>, CO, O<sub>3</sub>, and NO<sub>2</sub> were used in individual models. Post hoc pairwise comparisons with Tukey adjustment for multiple comparisons were used in the case of categorical AQI. Analyses were performed using the lme4 package in R, version 4.2.3 (The R Foundation), and a *P* value of < 0.05 was considered statistically significant.<sup>17</sup>

## Results

### Dogs

A total of 15 dogs were enrolled in the study; however, not all dogs were available for each air quality index category due to scheduling reasons. The number of dogs for each period was as follows: good (fall 2022) = 15 dogs, good (spring 2023) = 14 dogs, moderate (fall 2022) = 15 dogs, and unhealthy for sensitive groups (smoke) (spring 2023) = 7 dogs. The median age of the dogs enrolled was 4.4 years (range, 1 to 7 years). There were 9 spayed females and 6 neutered males. There were 4 breeds represented with the most common being the mixed-breed dog (n = 11), followed by the Labrador Retriever (2), the Norwich Terrier (1), and the Jack Russell Terrier (1).

### Air quality parameters

Dogs were examined at 4 different time periods corresponding to 3 different AQI categories. The mean ± SD for the good (fall) period (October 6, 2022, to October 11, 2022) was 44.47 ± 3.03; for the good (spring) period (April 7, 2023, to April 12, 2023), it was 43.64 ± 0.69; for the moderate period (September 9, 2022, to September 19, 2022), it was 73.67 ± 4.58; and for the unhealthy for sensitive groups (smoke) period (May 21, 2023), it was 103.67. Wildfires burning in western Canada caused the AQI to reach the smoke category for Larimer County in May 2023.<sup>18</sup>

The mean ± SD for PM<sub>2.5</sub> for each period was as follows: good (fall), 9.16 ± 2.16 µg/m<sup>3</sup>; good (spring), 5.64 ± 0.46 µg/m<sup>3</sup>; moderate, 13.14 ± 1.01 µg/m<sup>3</sup>; and smoke, 32.80 µg/m<sup>3</sup>. The mean ± SD for CO for each period was as follows: good (fall), 0.35 ± 0.02 ppm; good (spring), 0.29 ± 0.03 ppm; moderate, 0.43 ± 0.06 ppm; and smoke (spring), 0.47 ppm. The mean ± SD for O<sub>3</sub> for each period was as follows: good (fall), 0.04 ± 0.003 ppm; good (spring), 0.05 ± 0.002 ppm; moderate 0.05 ± 0.006 ppm; and smoke, 0.06 ppm. The mean ± SD for NO<sub>2</sub> for each period was as follows: good (fall), 12.64 ± 3.42 ppb; good (spring), 14.07 ± 3.35 ppb; moderate 11.23 ppb; and smoke, 9.67 ppb. The mean ± SD for humidity for each period was as follows: good (fall), 60.5 ± 17.77%; good (spring), 22.2 ± 10.83%; moderate 47.9 ± 32.83%; and smoke, 20.1 ± 1.41%.

### Schirmer tear test-1

The mean ± SD for STT-1 combined for both eyes examined for each period was as follows: good (fall), 28.43 ± 3.96 mm/min; good (spring), 28.21 ± 3.17 mm/min; moderate, 28.7 ± 4.22 mm/min; and smoke, 29 ± 4 mm/min (**Figure 1**). There were no statistically significant relationships at the 0.05 level to air quality or environmental variables to STT-1. The following are the *P* values corresponding to the different environmental values: AQI (categorical), *P* = .794; AQI (numerical), *P* = .463; PM<sub>2.5</sub>, *P* = .489; CO, *P* = .427; O<sub>3</sub>, *P* = .292; NO<sub>2</sub>, *P* = .955; and humidity, *P* = .671.

### Intraocular pressure

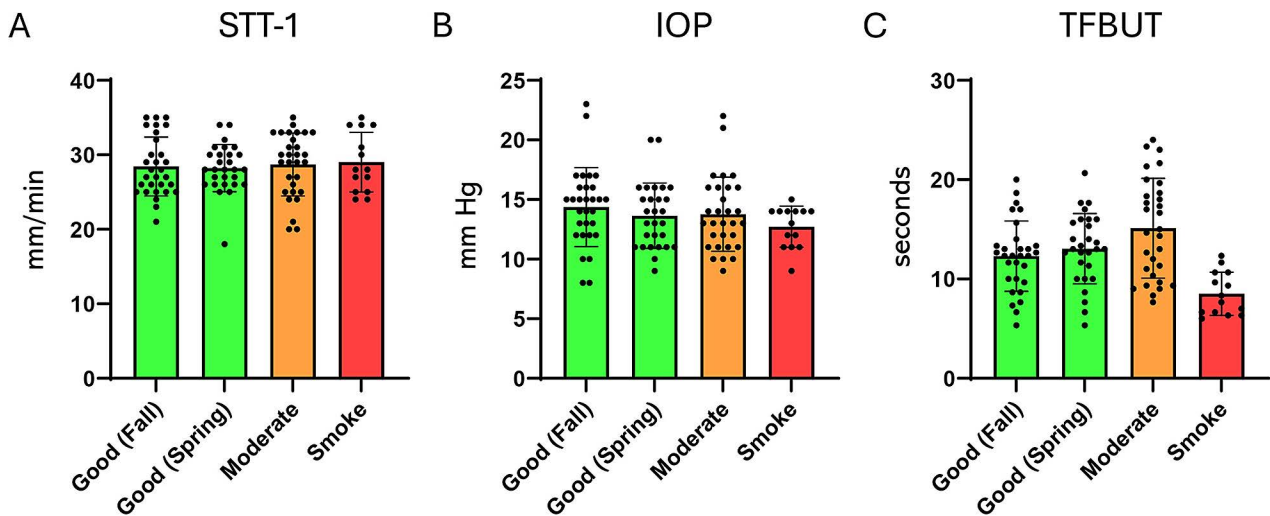
The mean ± SD for IOP combined for both eyes examined for each period was as follows: good (fall), 14.4 ± 3.32 mm Hg; good (spring), 13.6 ± 2.74 mm Hg; moderate, 13.8 ± 3.11 mm Hg; and smoke, 12.7 ± 2.74 mm Hg (Figure 1). There were no statistically significant relationships at the 0.05 level to air quality or environmental variables to IOP. The following are the *P* values corresponding to the different environmental values: AQI (categorical), *P* = .782; AQI (numerical), *P* = .368; PM<sub>2.5</sub>, *P* = .337; CO, *P* = .330; O<sub>3</sub>, *P* = .159; NO<sub>2</sub>, *P* = .182; and humidity, *P* = .137.

### Tear film break-up time

The mean ± SD for TFBUT combined for both eyes examined for each period was as follows: good (fall), 12.30 ± 3.54 seconds; good (spring), 13.05 ± 3.54 seconds; moderate, 15.11 ± 5.02 seconds; and smoke, 8.52 ± 2.17 seconds (Figure 1). There were no statistically significant relationships at the 0.05 level to air quality or environmental variables to TFBUT. The following are the *P* values corresponding to the different environmental values: AQI (categorical), *P* = .200; AQI (numerical), *P* = .368; PM<sub>2.5</sub>, *P* = .337; CO, *P* = .330; O<sub>3</sub>, *P* = .159; NO<sub>2</sub>, *P* = .182; and humidity, *P* = .884.

### Hyperemia

The mean ± SD for hyperemia score combined for both eyes examined for each period was as follows: good (fall): 0.33 ± 0.55; good (spring): 0.32 ± 0.45; moderate: 0.27 ± 0.45; and smoke: 0.86 ± 0.66. There



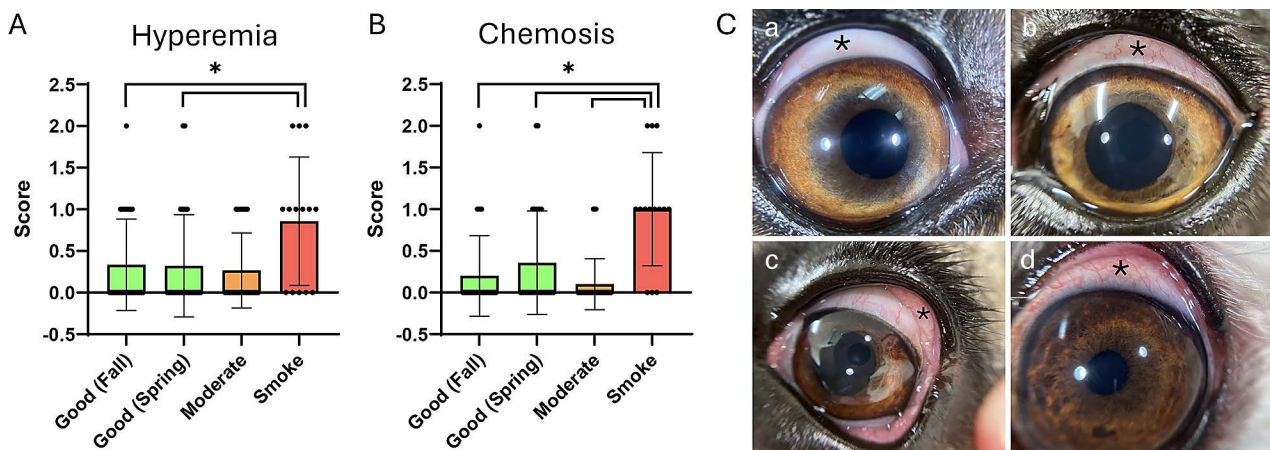
**Figure 1**—Mean Schirmer tear test-1 (STT-1; A), intraocular pressure (IOP; B), and tear film break-up time (TFBUT; C) of both eyes and SD bar for the cohort of dogs during each air quality index category period: good (fall), good (spring), moderate (fall), and unhealthy for sensitive groups (smoke). Each graph has corresponding error bars of SD.

was a statistically significant relationship between categorical AQI to the odds of having a higher level of hyperemia ( $P = .034$ ). There was an OR of 5.393, meaning the odds of having a higher level of hyperemia were 5.39 times higher in the smoke air quality category compared to the good category (**Figure 2**). There were no other statistically significant relationships between hyperemia and environmental variables. The following are the  $P$  values corresponding to the different environmental values: AQI (numerical),  $P = .422$ ;  $PM_{2.5}$ ,  $P = .068$ ;  $CO$ ,  $P = .574$ ;  $O_3$ ,  $P = .632$ ;  $NO_2$ ,  $P = .550$ ; and humidity,  $P = .721$ .

### Chemosis

The mean  $\pm$  SD for chemosis score combined for both eyes examined for each period was as follows:

good (fall),  $0.47 \pm 0.48$ ; good (spring),  $0.36 \pm 0.62$ ; moderate,  $0.1 \pm 0.31$ ; and smoke,  $1.0 \pm 0.68$ . There were 2 statistically significant relationships between categorical AQI and  $PM_{2.5}$  to the odds of having a higher level of chemosis ( $P = .026$  and  $P = .018$ , respectively). The odds of having a higher level of chemosis were 7853.2 times higher during the smoke AQI category than the good AQI category. For every additional point of  $PM_{2.5}$ , the odds of a higher level of chemosis were multiplied by 1.105 (Figure 2). There were no other statistically significant relationships between chemosis and environmental variables. The following are the  $P$  values corresponding to the different environmental values: AQI (numerical),  $P = .278$ ;  $CO$ ,  $P = .600$ ;  $O_3$ ,  $P = .266$ ;  $NO_2$ ,  $P = .753$ ; and humidity,  $P = .393$ .



**Figure 2**—A and B—Graphs of the mean scores (both eyes) with SD bar for hyperemia (A) and chemosis (B) during each air quality index category period: good (fall), good (spring), moderate (fall), and unhealthy for sensitive groups (smoke). \*Statistical significance. C—Images of dogs that were involved in the present study at various time periods demonstrating various levels and corresponding grading scores for hyperemia and chemosis. a—Eye with no signs of hyperemia or chemosis (score 0/3). b—Mild hyperemia and chemosis (score 1/3). c—Mild hyperemia (score 1/3) and moderate chemosis (score 2/3). d—Severe hyperemia (score 3/3) and moderate chemosis (score 2/3).

## Bacterial culture

The percentage of dogs that yielded microbial growth upon aerobic culture for each period was as follows: good (fall), 67% (10/15); good (spring), 50% (7/14); moderate, 53% (8/15); and smoke 29% (2/6). The mean  $\pm$  SD of different bacterial species and in a few cases, fungal species, identified for each period was as follows: good (fall),  $2.33 \pm 2.85$ ; good (spring),  $1.21 \pm 1.42$ ; moderate,  $1.73 \pm 2.09$ ; and smoke,  $0.57 \pm 0.98$ . There were no statistically significant relationships between whether there were bacteria isolated and environmental variables. The following are the  $P$  values corresponding to the different environmental values: AQI (categorical),  $P = .415$ ; AQI (numerical),  $P = .249$ ;  $PM_{2.5}$ ,  $P = .275$ ; CO,  $P = .890$ ;  $O_3$ ,  $P = .382$ ;  $NO_2$ ,  $P = .727$ ; and humidity,  $P = .739$ .

The full list of microbiota isolated from each dog at the various air quality periods is provided (**Supplementary Table S1**). For the good (fall) period, there were a total of 34 different organisms identified. The mean  $\pm$  SD for humidity for this time period was  $60.5 \pm 17.8\%$ . The most frequently isolated organisms were coagulase-negative *Staphylococcus* spp ( $n = 5$ ), gram-negative nonfermenters that were not speciated (4), as well as *Bacillus* spp (3), *Streptococcus* spp alpha-hemolytic (3), and *Streptomyces* spp (3). For the good (spring) period, there were a total of 16 different organisms identified via culture. The mean  $\pm$  SD for humidity for this time period was  $22.2 \pm 10.8\%$ . The most frequently identified species were coagulase-negative *Staphylococcus* ( $n = 5$ ), coryneform bacteria that were not speciated (3), as well as *Bacillus* spp (2) and *Rothia* spp (2). For the moderate period, there were a total of 22 different organisms identified via culture. The mean  $\pm$  SD for humidity for this time period was  $47.9 \pm 32.8\%$ . The most frequently identified species were coagulase-negative *Staphylococcus* spp ( $n = 3$ ), coryneform bacteria that were not speciated (3), as well as *Arthrobacter* spp (2), *Bacillus* spp (2), *Staphylococcus pseudintermedius* (2), *Streptococcus* spp alpha hemolytic (2), and *Streptomyces* spp (2). For the smoke period, there were a total of 4 different organisms identified via culture. The mean  $\pm$  SD for humidity for this time period was  $20.1 \pm 1.4\%$ . Each of the following species was represented once: *Neisseria* spp, *Rothia* spp, *Staphylococcus pseudintermedius*, and *Streptococcus* spp nonhemolytic.

## Discussion

Changing environmental factors have led to increased severity and lengthening of fire seasons globally.<sup>1-3</sup> Smoke affects large areas that are not necessarily adjacent to large wildfires. National Aeronautics and Space Administration satellite images have demonstrated that smoke plumes travel thousands of miles stretching across the entire US.<sup>19</sup> While the effects of smoke inhalation on the cardiovascular and pulmonary systems in humans have been relatively well studied, few have specifically investigated the effects of wildfire smoke on the ocular surface.<sup>20-22</sup> In the present study, we found a

statistically significant relationship between higher categorical AQI and a higher chemosis ( $P = .018$ ) and hyperemia scores ( $P = .034$ ), indicating conjunctivitis and ocular irritation are related to decreased air quality. This is supported by studies<sup>4,23</sup> in humans that have identified decreased TFBUTs and symptoms including itching, burning, and a foreign body sensation due to wildfire smoke exposure. The same clinical signs in dogs can lead to scratching and rubbing and the formation of corneal trauma and ulcerations.<sup>24</sup>

The present study did not demonstrate any statistically significant relationships between diagnostic tests evaluating the tear film (TFBUT and STT) and decreased air quality; however, the increased chemosis and hyperemia scores indicate some level of ocular surface irritation or inflammation. Although not statistically significant, the mean  $\pm$  SD TFBUT for the smoke category was  $8.52 \pm 2.17$  seconds compared to the other categories with a mean TFBUT between 12.30 to 15.11 seconds. The low TFBUT for the smoke category was found after only a 3-day exposure to AQI above 100 due to wildfire smoke. This low TFBUT could potentially be clinically relevant for these dogs and may decrease even more if the dogs were exposed to wildfire smoke for a longer period. This corresponds to human studies<sup>4</sup> regarding TFBUT and wildfire smoke. The TFBUT is a noninvasive way to assess tear film stability in both humans and animals. There is a relatively large range of what is normal as many variables can affect the results of the test, such as subjective interpretation and variation in fluorescein instilled, but the TFBUT in healthy dogs is established to be approximately 20 seconds, with a range of 15 to 40 seconds.<sup>15</sup> The mean TFBUT for all the categories tested was  $12.25 \pm 2.75$  seconds, below the previously established normal in dogs, which could be a consequence of Colorado's arid climate causing a decrease in tear film stability overall, and therefore, established normal reference ranges for TFBUT may need to account for environmental factors. A possible confounding factor however could be related to conjunctival irritation from the microbiologic culture sample causing an artificial decrease in TFBUT. Further investigation is warranted regarding the effects of manual irritation before performing TFBUT.

Fine PM, particles with an aerodynamic  $PM_{2.5}$ , are identified in wildfire smoke and potentially pose the biggest risk to public health.<sup>7,20-22,25</sup> One study<sup>7</sup> revealed that  $PM_{2.5}$  specifically from wildfires was up to 10 times more harmful to human health than  $PM_{2.5}$  from other sources. In support of this, our research found an association between  $PM_{2.5}$  and chemosis score, as a higher average  $PM_{2.5}$  increased the odds of having a higher level of chemosis (OR = 1.174;  $P = .027$ ). Although the reasoning behind the increased toxicity of wildfire  $PM_{2.5}$  compared to other sources is not completely understood, it is postulated that the higher carbon content may contribute to a higher level of oxidative potential and corresponding oxidative stress and inflammation.<sup>7</sup> This could be hypothesized as the underlying mechanism causing

an increased level of chemosis in the dogs examined in this study during higher PM<sub>2.5</sub> exposure.

Wildfire smoke has also been associated with an increase in O<sub>3</sub> in ambient air.<sup>7</sup> Ozone is another potential oxidant shown to increase the risk of hospitalizations for respiratory conditions and has been linked to worsening symptoms in patients with dry eye disease.<sup>25,26</sup> Although the present study did not identify any statistically significant relationships between any of the ocular surface parameters and O<sub>3</sub> levels, it should continue to be evaluated in future studies looking at the effects of air quality and smoke exposure on the eyes.

Our previous study<sup>10</sup> demonstrated a significant increase in dogs presenting with infected corneal ulcerations during the 2020 Colorado wildfires and identified some unusual bacterial isolates including *Mycobacterium fortuitum*. Wildfires are a potential source of bioaerosols: aerosolized bacteria, fungi, and their metabolic bioproducts.<sup>27</sup> It was postulated that these microorganisms had the potential to get in the eye and contribute to infectious keratitis in dogs. For this reason, the present study aimed to evaluate the conjunctival flora in relation to decreased air quality and wildfire smoke exposure. There was no significant increase in the percentage of positive cultures corresponding to different AQI categories evaluated. The most commonly identified organisms in all of the categories were coagulase-negative *Staphylococcus spp*, which is consistent with previous reports<sup>28</sup> that *Staphylococcus* and other gram-positive bacterial genera (*Streptococcus spp* and *Bacillus spp*) are the most common ocular surface bacteria in dogs. An interesting pattern identified in this study was that during times of higher humidity, the moderate (mean  $\pm$  SD; 47.9  $\pm$  32.8%) and good (fall) (60.5  $\pm$  17.77%) periods, there was a higher number of microbial species identified, 22 and 34, respectively. There was not a statistically significant relationship between humidity and positive microbial culture. However, this may indicate more of a relationship between humidity and ocular flora than other ambient air substances. High humidity has been linked to an increased incidence of infectious keratitis in humans and horses<sup>29,30</sup>; the present study may demonstrate an increase in conjunctival bacteria corresponding to times of higher ambient moisture.

The American Academy of Ophthalmology in 2020 published recommendations online of how humans can find relief from ocular smoke irritation.<sup>31</sup> Similar recommendations can be made for our companion animals during times of smoke exposure to reduce the clinical signs and the potential for corneal ulceration formation. Staying indoors as much as possible and using air filters can help decrease exposure to PM and other harmful substances in smoke. When they must be outside, if they can tolerate it, pets can wear goggles to help protect their eyes. To help with conjunctivitis and potential tear film instability artificial tears can be applied and kept in the refrigerator.<sup>31</sup>

A limitation of this and other prospective wildfire studies is that exposure cannot be planned. The

2022 Colorado wildfire season was predicted to be the worst wildfire season of the state's history, due to the severe drought classification and fast depletion of the season's snowpack.<sup>32</sup> Fortunately, for those living in Colorado this did not come to light. However, this meant that, during the study period, there was only one time when the AQI surpassed 100 getting into the unhealthy for sensitive groups category. Another limitation of this study was the sample size, which contributed to a low statistical power. Due to scheduling difficulties, all the dogs were unable to be evaluated at all 4 of the time periods, which most significantly limited the smoke category. Another variable not evaluated in this study would be potential allergen exposure (plant pollen). This is not accounted for in the AQI score, varies at different periods throughout the year, and has the potential to cause allergic conjunctivitis in dogs.<sup>24</sup>

In conclusion, an increase in AQI due to wildfire smoke led to conjunctivitis in dogs. Particulate matter < 2.5  $\mu$ m in diameter seemed to be associated with an inflammatory factor, which is a known component in wildfire smoke. Conjunctivitis in dogs can cause itching, rubbing, and corneal trauma, leading to painful and potentially site-threatening infected corneal ulcerations. As the risk of wildfires continues to increase globally, it is important to take these clinical signs seriously and follow preventive recommendations.

## Acknowledgments

The authors acknowledge all of the Colorado State University's students and employees who were involved in this study for their assistance and participation.

## Disclosures

The authors have nothing to disclose. No AI-assisted technologies were used in the generation of this manuscript.

## Funding

Funding was provided by the Kenneth W. Smith Professorship in the Center for Companion Animal Studies (Colorado State University, Fort Collins, CO).

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## Supplementary Materials

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