A pilot study on the lung morphology of shelter dogs in relation to air pollution in Trinidad

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OBJECTIVE
To identify and characterize the gross and histological lesions associated with air pollution in the lungs of dogs from various locations in Trinidad.

ANIMALS
56 fresh lungs were obtained from already euthanized adult dogs collected from different locations in Trinidad at the Trinidad and Tobago Society for the Prevention of Cruelty to Animals.

PROCEDURES
Lung specimens were examined grossly and tissue samples were taken for routine histologic examination.

RESULTS
Histological examination showed that 51.8% of the dogs had evidence of anthracosis. Dogs with anthracosis had greater median lesion scores compared to dogs without anthracosis (P = .022). There was no association between the presence of anthracosis and any other lesion in this study (P > .05).

CLINICAL RELEVANCE
There was evidence that dogs with anthracosis had a greater degree of nonspecific lung histologic lesions. Using the dog as a sentinel model for human exposure in Trinidad, our findings indicate that environmental air pollution may also have an effect on the respiratory health of the human population. It is important for the public to be aware of air pollution, and the government of Trinidad and Tobago should develop an intervention protocol along with veterinary and human medical epidemiologists to reduce air pollution in the country.

Trinidad and Tobago is the most industrialized country in the Commonwealth Caribbean region and experiences the attendant environmental problems associated with the production of petrochemicals, uncontrolled urbanization, and motorization, which have a significant impact on air quality.1-3 With a population of just less than 1.4 million, there is an estimated 1 million motor vehicles on the nation’s roads.4,5 Black carbon emission concentrations near major roads in Trinidad reached levels comparable to those near highways in large Metropolitan cities. The elevated black carbon concentrations in Trinidad were likely a result of diesel exhaust, with significant contributions from motor vehicle traffic.6,7 Levels of particulate matter (PM) between PM2.5 and PM10, which exceed the World Health Organization safe-limit guidelines have also been detected in areas along Trinidad’s West coast, which are characterized as rural, urban, mixed background, and industrial land use.8 Gaseous pollutants have also been found to be high.9,10

Low levels of air pollution can affect the health of susceptible and sensitive people, especially those living in large urban areas, where road emissions are a contributing factor.11-12 The clinical effects of air pollution include cough, shortness of breath, wheezing, asthma, respiratory disease, hospitalization, pulmonary insufficiency, cardiovascular diseases, and neoplasia.12-14 A Swedish study15 indicated air pollution may also induce diabetes.

Animals that live in polluted environments can provide important biologic data for assessing possible risks to human health. Wild city pigeons can be used as biologic indicators of exposure to heavy metal pollution in outdoor air.6 The lungs of dogs and humans share similarities in terms of size, anatomy, patterns of development, pulmonary function, and cellular composition.16 The life span of dogs is sufficiently long for inclusion in longitudinal studies. Therefore, canines are often chosen as an experimental model or sentinel species for the study of pulmonary responses to long-term exposure to air pollution.
pollutants.\textsuperscript{17} Stray dogs that roam the streets are exposed to air pollutants—especially those from vehicle exhaust—and, therefore, changes in their lungs are a good indicator of the potential effects of air pollution.

Histologic changes in the lungs of street dogs in Mexico surveyed as sentinels for human exposure included mononuclear cell infiltrates along with macrophages loaded with PM surrounding the bronchial walls and extending into adjacent vascular structures, bronchiolar epithelial and smooth muscle hyperplasia, peribronchiolar fibrosis, microthrombi, and capillary and venule polymorphonuclear leukocyte margination.\textsuperscript{16} Histologic changes that can also occur in the lungs of dogs with exposure to air pollution include enlargement of air spaces, nonspecific inflammation, accumulation of alveolar macrophages, thickening of bronchiolar walls, squamous metaplasia, and deposition of fibrous elements.\textsuperscript{17}

Lungs are the primary organ at risk from inhalation of carbon particles and asbestos dust.\textsuperscript{18} Anthracosis is caused by carbon accumulation in the lungs resulting from inhalation of coal dust particles and smoke, or exposure to air pollution. It occludes the bronchial lumen of the lungs in the form of black discoloration and is associated with bronchial anthracofibrosis.\textsuperscript{19} Anthracosis was found in the lung tissue of domestic and wild animals.\textsuperscript{20} With Trinidad being a relatively industrialized country with the known presence of air pollution as well as having a small stray dog population, we believe that assessment of the lungs of stray dogs will provide an indication of the effects that air pollution has on dogs as well as humans. The objective of this study was to identify and characterize the gross and histologic lesions associated with air pollution in the lungs of dogs from various locations in Trinidad.

\section*{Materials and Methods}

Ethical approval was granted by The University of the West Indies, Campus Ethics Committee (CEC 181/04/17). A total of 56 dogs were sourced from the Trinidad and Tobago Society for the Prevention of Cruelty to Animals shelter. The dogs were taken from the following known locations: Sangre Grande, San Juan, Chaguana, Port-of-Spain, San Fernando, St. James, Cunupia, Diego Martin, and Santa Cruz. The dogs were examined by a veterinarian before they were euthanized via IV infusion of 100 mg/kg sodium pentobarbital (Akorn, Inc) via the cephalic vein. The entire lungs and trachea were eviscerated from the thorax.

Specimen analyses were conducted by personnel of the Anatomy and Pathology Laboratories of the School of Veterinary Medicine, Faculty of Medical Sciences. Topographic position of the lungs in situ was recorded, then the lungs were removed from the thoracic cavity. Gross examination of the lungs was performed for the presence of anthracosis, and photographs were taken using a Sony 12 MP digital camera (Figure 1).

For microscopic examination, a minimum of 4 sections of the left and right lungs, including the cranioventral and caudodorsal areas from each side, were removed and placed in 10% neutral formaldehyde. Formalin-fixed tissues were processed according to standard methods, cut with a microtome (Shandon Finesse ME) at 4 μm, and stained with H&E. The slides were examined by a trained veterinary pathologist using an Olympus BX-40 microscope. Images of lesions were taken with an Olympus DP 71 megapixel digital camera. Sections with visible fibrosis and smooth muscle hyperplasia on H&E staining were stained further with Masson's trichrome (MASTERTECH) and elastin (MASTERTECH) stains, respectively, according to the manufacturer's instructions. The slides were examined and assessed for anthracosis as well as other lesions that may be associated with air pollution, including fibrosis, inflammation/pneumonia, enlargement of air spaces, and thickening of bronchiolar walls.\textsuperscript{17,18,21}

For statistical analysis, histopathologic lung lesions were scored subjectively, and the sum of the scores for nonspecific lesions was calculated (Tables 1 and 2). Dogs were categorized into those with and without anthracosis. The lesion scores for the 2 groups were subjected to the Kolmogorov-Smirnov test for normality. The difference in median lesion scores for the 2 groups was determined using the Mann Whitney test. The χ² test for independence with the Yate's continuity correction was
Table 1—Guidelines for grading histological changes

| Lesion                  | Within normal limits (0 point) | Mild (1 point) | Moderate (2 points) | Severe (3 points) |
|-------------------------|--------------------------------|----------------|---------------------|-------------------|
| Anthracosis             | None                          | Occasional to multiple small foci | Multiple large foci | > 30% of the lung |
| Air space enlargement   | None                          | Mild expansion of < 10/hpf        | Mild to moderate expansion of 11 to 20/hpf | Moderate to severe expansion of > 20/hpf |

hpf = High-power field.

Table 2—Microscopic lesions of lungs of stray dogs in Trinidad

| Dog no. | Anthracosis | Fibrosis | Pneumonia | Enlargement of air spaces | Thickening of alveolar wall | Total score (nonspecific lesions) |
|---------|-------------|----------|-----------|---------------------------|-----------------------------|----------------------------------|
| 112-18  | 2           | 1        | 1         | 1                         | 1                           | 4                                |
| 113-18  | 1           | 1        | 2         | 1                         | 2                           | 6                                |
| 185-18  | 0           | 0        | 0         | 0                         | 0                           | 1                                |
| 246-18  | 1           | 1        | 1         | 2                         | 1                           | 5                                |
| 247-18  | 1           | 0        | 0         | 2                         | 2                           | 4                                |
| 249-18  | 1           | 0        | 0         | 1                         | 2                           | 6                                |
| 200     | 0           | 0        | 0         | 1                         | 2                           | 4                                |
| 201     | 0           | 0        | 0         | 1                         | 2                           | 4                                |
| 300     | 1           | 1        | 1         | 1                         | 2                           | 5                                |
| 301     | 0           | 0        | 2         | 1                         | 2                           | 5                                |
| 302     | 0           | 0        | 2         | 1                         | 2                           | 5                                |
| 303     | 1           | 1        | 1         | 1                         | 2                           | 5                                |
| 304     | 0           | 0        | 2         | 0                         | 1                           | 5                                |
| 305     | 0           | 0        | 2         | 2                         | 1                           | 5                                |
| 306     | 0           | 0        | 0         | 0                         | 0                           | 0                                |
| 400     | 1           | 0        | 0         | 0                         | 0                           | 0                                |
| 401     | 1           | 1        | 1         | 0                         | 0                           | 0                                |
| 402     | 1           | 1        | 2         | 1                         | 1                           | 5                                |
| 500     | 1           | 0        | 1         | 1                         | 1                           | 5                                |
| 501     | 0           | 0        | 1         | 0                         | 0                           | 0                                |
| 502     | 1           | 0        | 1         | 1                         | 1                           | 3                                |
| 503     | 0           | 0        | 1         | 1                         | 1                           | 3                                |
| 504     | 1           | 0        | 1         | 1                         | 1                           | 3                                |
| 505     | 0           | 0        | 1         | 1                         | 1                           | 3                                |
| 600     | 1           | 1        | 0         | 1                         | 1                           | 5                                |
| 601     | 1           | 0        | 0         | 0                         | 0                           | 0                                |
| 700     | 1           | 0        | 1         | 0                         | 0                           | 0                                |
| 701     | 0           | 0        | 1         | 2                         | 1                           | 5                                |
| 702     | 1           | 0        | 1         | 1                         | 1                           | 3                                |
| 800     | 1           | 0        | 1         | 1                         | 1                           | 3                                |
| 801     | 1           | 0        | 1         | 1                         | 1                           | 3                                |
| 802     | 0           | 1        | 1         | 1                         | 1                           | 5                                |
| 803     | 0           | 0        | 2         | 1                         | 1                           | 5                                |
| 804     | 1           | 0        | 1         | 1                         | 1                           | 5                                |
| 805     | 1           | 1        | 0         | 1                         | 1                           | 5                                |
| 806     | 1           | 0        | 0         | 2                         | 1                           | 5                                |
| 807     | 0           | 0        | 0         | 1                         | 1                           | 3                                |
| 808     | 0           | 0        | 0         | 1                         | 1                           | 3                                |
| 900     | 0           | 0        | 0         | 1                         | 1                           | 1                                |
| 901     | 1           | 1        | 1         | 1                         | 1                           | 3                                |
| 902     | 0           | 0        | 1         | 1                         | 1                           | 3                                |
| 903     | 0           | 0        | 0         | 1                         | 0                           | 2                                |
| 904     | 1           | 0        | 0         | 2                         | 1                           | 5                                |
| 905     | 0           | 0        | 0         | 1                         | 1                           | 3                                |
| 906     | 0           | 0        | 0         | 1                         | 1                           | 3                                |
| 907     | 0           | 0        | 0         | 1                         | 1                           | 3                                |
| 908     | 0           | 0        | 0         | 1                         | 1                           | 3                                |
| 909     | 0           | 0        | 0         | 1                         | 1                           | 3                                |
| 105     | 1           | 0        | 1         | 0                         | 0                           | 1                                |
| 1001    | 0           | 0        | 1         | 0                         | 0                           | 1                                |
| 1002    | 1           | 0        | 2         | 1                         | 2                           | 5                                |
| 1003    | 0           | 0        | 1         | 1                         | 1                           | 3                                |
| 1004    | 0           | 0        | 1         | 1                         | 1                           | 3                                |
| 1005    | 1           | 0        | 1         | 1                         | 1                           | 3                                |
| 3000    | 0           | 0        | 1         | 1                         | 1                           | 3                                |
used to determine whether there was an association between the presence of anthracosis and other histologic findings such as pneumonia, thickening, or fibrosis. Data were analyzed using SPSS version 24 (IBM Corp). Statistical significance was set at $P \leq .05$.

Results

Gross observations

Lesions of anthracosis—characterized as multiple, small (< 1-mm-diameter) pinpoint black foci scattered through the lung (Figure 2)—were found in 2 dogs (3.6%), predominantly on the cranial and middle lobes, occupying approximately 10% of the parenchyma. Focal areas of hemorrhage 2 to 8 mm in diameter were seen in the lungs of 18 dogs (32.1%), particularly in the right middle and accessory lobes.

Microscopic observations

The histologic lesions were graded subjectively as follows: within normal limits, 0 point; mild, 1 point; moderate, 2 points; and severe, 3 points (Table 1). The results for histologic lesions in the lungs were tabulated (Table 2). Anthracosis is associated with air pollution, whereas the other lesions are nonspecific.

Histologic lesions of anthracosis were found in the lungs of 29 dogs (51.8%). Carbon was found primarily intracellularly in macrophages forming granulomas, predominantly close to the bronchiolar-associated lymphoid tissue and in the lungs of dogs. Microscopic granulomas were also scattered in the alveolar interstitium (Figures 3 and 4). The nonspecific lesions included chronic interstitial pneumonia in 54 dogs (96.4%), enlargement of the air spaces (Figure 2) in 46 dogs (82.1%), thickening of the

Figure 2—Multiple < 1-mm-diameter pinpoint black foci (A) and focal areas of hemorrhage (B).

Figure 3—A—Small numbers of inflammatory cells in the alveolar interstitium (yellow arrowheads) indicating a mild interstitial pneumonia. At 40X magnification, H&E stain. B—The alveolar interstitium is expanded by moderate numbers of inflammatory cells (yellow arrowheads) suggestive of a moderate interstitial pneumonia. At 40X magnification, H&E stain. C—Multifocal granulomas (yellow arrowheads) composed predominantly of macrophages containing carbon particles (anthracosis) and enlargement of air spaces (blue arrowheads). At 20X magnification, H&E stain. D—Smooth muscle hyperplasia (blue arrowheads) around a bronchiole (B). At 20X magnification, H&E stain.
Lesions score totals were between 0 and 6 points, with a median score of 3 points for dogs without anthracosis, and between 1 and 6 points for dogs with anthracosis. This difference was statistically significant ($P = .022$).

**Discussion**

Stray dogs live on city streets and are exposed continuously to emissions from vehicular traffic as they remain on the streets throughout the day, whereas most people may only be exposed during peak commuting hours. Other persons, such as the homeless, those who work outdoors, and street vendors, would likely have increased exposure to air pollution compared to the general population. Dogs are also closer to the ground and it is more likely that smaller PM would enter their lungs. A smog incident caused illness and mild respiratory problems and also a few deaths in Pennsylvania.23 A recent report24 stated that dogs with chronic bronchitis and cats with airway inflammatory disease are at increased risk of exacerbating their conditions if exposed to prolonged urban air pollutants, similar to humans. The authors recommended suppression of the ongoing inflammatory processes by medical therapy and avoidance of exercising pets outdoors in urban areas during peak pollutant periods.

The histologic detection of anthracosis as found in the lungs of 29 dogs (51.8%) indicates the presence of carbon particles in the air. The carbon particles were typically found mainly in granulomas with varying severity. Gross lesions were demonstrated in the lungs of 2 dogs (3.8%). Anthracosis present on its own, however, would not likely have been sufficient to result in respiratory compromise. The level of anthracosis found supports the finding that black carbon is an important air pollutant in Trinidad.7

Air pollution, especially indoor air pollution, can also produce disease in dogs. Cigarette smoke inhalation can result in anthracosis and the development of illnesses such as nasal cavity and lung cancer, dermatitis, and respiratory disease.25–28 Poor indoor air quality can exacerbate airway disease in pets.29 The dogs surveyed in our study were all outdoor strays; therefore, it is likely that the anthracosis was caused by air pollution.

The histologic lesions were also similar to those found in air pollution exposure studies in laboratory rats.17,18,21 Calderón-Garcidueñas et al36 used a somewhat different classification system; however, PM in mononuclear phagocytes would be similar to anthracosis. Dilation of the terminal and respiratory bronchioles could correlate to the expansion of the air spaces noted in our study. Fibrosis was not limited to peribronchiolar areas in the dogs in our study. In the study from Mexico City,24 most of the lesions had a high level of occurrence, with many being more than 78%, and with interstitial pneumonia (96.4%) and enlargement of the air spaces (82.1%) occurring at similar rates in our study. Prolonged exposure of dogs with chronic bronchitis to urban air pollutants can exacerbate their conditions.24

Ours is the first study to demonstrate the lesions associated with air pollution in stray dogs in Trinidad. The domestic dog can serve as an indicator species to assess environmental health and pollution. These dogs were predominantly strays, which were collected from urban areas and therefore would have been exposed to air pollutants. The small island of Trinidad, with a population of just more than 1.3 million, has approximately 1 million registered vehicles,
which most likely contributes to air pollution.\textsuperscript{1,5} Anthracosis in the lungs of these dogs indicates the inhalation of carbon particles. Therefore, air pollution is likely to have played a role in the lesions in the lungs of these dogs, many of which were typical for air pollution.

The finding of anthracosis and other lesions in dogs strongly suggests that air pollutants are present in Trinidad and may pose a health risk to both animals and humans. Government authorities should therefore devote more resources to monitor and control air pollution, especially in areas where there is prolonged or increased levels of exposure for humans and animals. Concurrent measurement of the levels of PM and other common air pollutants, and documentation of the exact location of the indicator animals used as well as ultrastructural evaluation of the lesions will add to more in-depth understanding of the lesions and the potential impact on the general population using the dog as an indicator species.

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