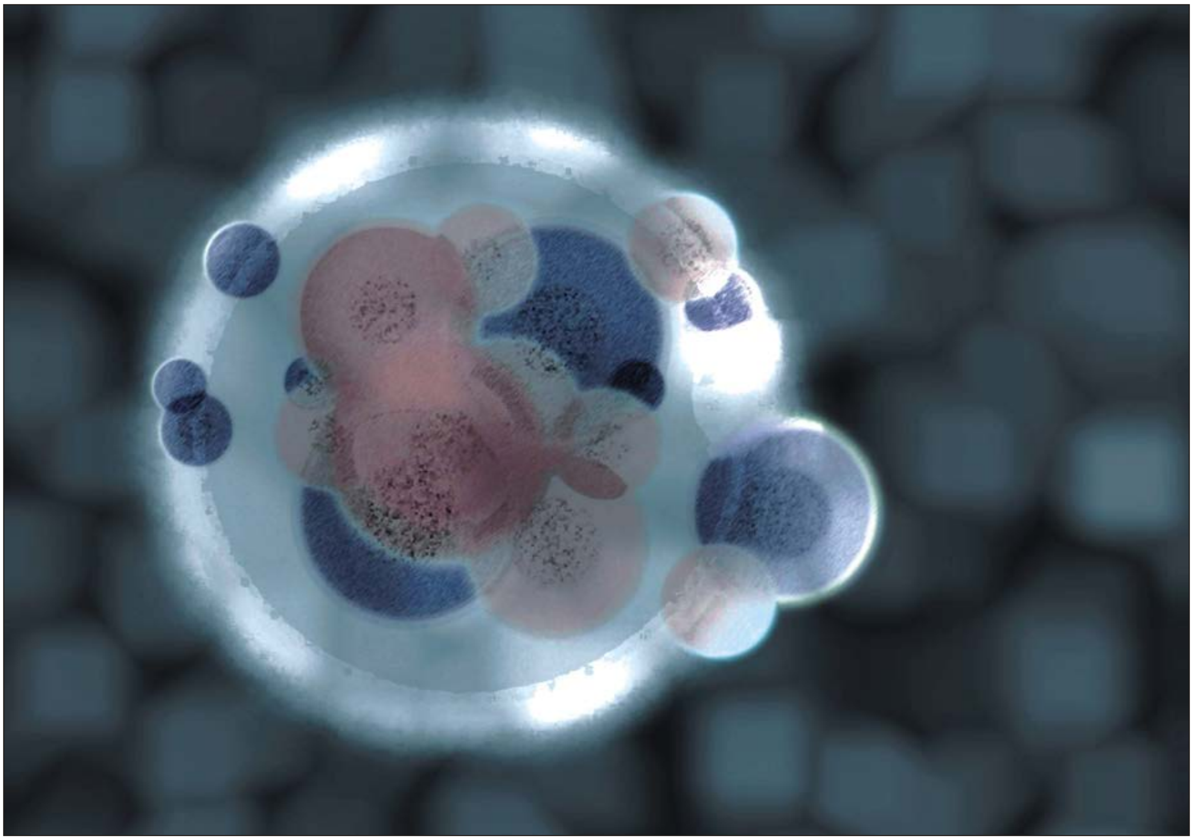

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Energy Dispersive X-ray Microanalysis of Dust Particles in Equine Stable

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Abstract: This study was conducted to evaluate the characteristics of dust particles in equine stables at Equine Centre, Universiti Putra Malaysia. In this study, the size of settled dust particles ranged from 5 to 10 µm, while unsettled dust particles ranged between 1 and 5 µm. This present study noted that 66% of respirable dust were less than 5 µm. These particles are capable of impairing alveolar function. A detailed examination of the dust particles indicated that 15.11% were oval and elongated in shape. Other studies have document that oval and elongated particles were capable of penetrating the alveolar respiratory system. Variable pressure scanning electron microscope attached to energy dispersive x-ray (VPSEM-EDX) revealed that settled dust in this study comprise the elements carbon, oxygen, magnesium, aluminium, silicon, sulphur, potassium, calcium, iron, phosphorus, sodium and bromine. Carbon and oxygen were the largest components in dust at 78 and 17%, respectively. Unsettled dust (inhalable) have higher levels of magnesium, aluminium, silicon, sulphur, carbon and bromine. These elements are important because of its probable role in causing hypersensitivity and impairment to alveolar function.

Key words: Dust particles, horse, VPSEM-EDX, elements

INTRODUCTION

Of all organs, the respiratory tract presents the largest and most delicate surface for contact with potentially deleterious airborne material. The alveolar and bronchiolar areas, which in the adult horse approximate 100 m^2 at end expiration and 300 m^2 at end inspiration, are exposed to approximately 30 million L of air a complex mixture of gaseous and particulate pollutants annually[1].

The air borne dust present in equine stables is a variable and complex mixture of organic and inorganic contaminants. These include bacteria, viruses, moulds, mite debris and feces, plant material, bacterial endotoxins, β-glucans and inorganic dust. These agents are potentially able to induce airway inflammation, either by initiating infection, inducing allergy, direct toxicity or indirectly by overwhelming the pulmonary defence mechanism[2]. The most widely recognized disorder affecting adult horses is chronic obstructive pulmonary disease or recurrent airway obstruction, also termed as heaves[3]. In some studies, pharyngeal lymphoid hyperplasia has also been linked to inhalation of airborne dust[1].

Although the levels of dust recorded in horse stables are lower than in intensive livestock buildings, the horse is more sensitive than other species and the length of exposure is significantly greater[4]. Air quality studies in the stable have been mainly confined to the levels of dust and microorganism in stables and the effects of airborne dust on equine respiratory diseases[5].

In this study, the size of dust in an equine stable was quantified by VPSEM-EDX. Though quantifying dust particle using VPSEM-EDX is possible, it has never been previously reported.

MATERIALS AND METHODS

Samplings: Settled dust was sampled from the body of 5 horses randomly selected from the Equine Centre, Universiti Putra Malaysia. Unsettled dust was obtained vacuum using from 5 stables at the Equine Centre. Sampling was carried out at 9 am at the peak of work at the stables. A physical examination was carried out on all 5 horses sampled, prior to sampling.

Settled dust from face of horses: A clean toothbrush was used to brush dust from the dorsal surface of the equine's face. Dust was allowed to settle on the surface of a double-sided tape on a glass slide. It was then placed in a petri dish to prevent contamination by other foreign materials and quantified by using VPSEM-EDX.

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Dust vacuum from stable: A 1300 W household vacuum (National®) was used to vacuum the unsettled dust from the stable. The vacuum was turned on for 5 min at the level of the horse nostrils. Three filter papers of different pore sizes (Whatman®) (0.4 and 0.2 μm) were placed at the opening hose of the vacuum. At the end of 5 min, a glass slide with double-sided tape was gently touched on the filter paper and placed in petri dish (9 cm diameter) to prevent contamination. This was repeated with all sampling.

Analysis: The dust samples was analysed using a Variable Pressure Scanning Electron Microscope (VPSEM). Five fields from every slide were evaluated. The sizes of particles were determined using this method. Elements in the dust particles were also analysed and their shapes categorized.

RESULTS AND DISCUSSION

Unsettled dust in this study ranged between 1 μm to less than 5 μm. The mean size of the unsettled dust is 4.360±1.126 μm. While for the settled dust is ranged between 5 μm to less than 10 μm. The mean size of settled in this study is 9.546±0.862 μm. In inhalable unsettled dust, 66% of the particles are respirable (less than 5 μm). Whereas in settled dust, 18% are respirable.

The dusts in this study were categorized into spherical, oval and elongated and irregular shapes. From the study, 50.5 % of particles were irregular shape, 34.39% spherical shape and 15.11% oval and elongated.

Elements in both settled and unsettled dust are shown in Table 1, carbon and oxygen are the two main elements in dust. Unsettled dust appears to have higher fraction of Al, Si, Ca and Na.

Dust is mostly measured as mg (airborne dust) per cubic meter of airspace (mg m^{-3}). Its rate is a measure of the amount of dust released into the airspace per hour per animal or kg live weight of animal[5]. Dust also can estimated in terms of the number of particles present within the airspace[6]. Studies showed that it can ranged form 3.11 to 443.85 particles mL^{-1} for particles less than 1.0 μm in size, 19.27 to 553.12 particles mL^{-1} for particles less than 2.0 μm in size, from 2.28 to 166.28 particles mL^{-1} for particles less than 5.0 μm in size[1].

In this study, particle size of unsettled dust range between 1 to <5 μm comprises 66% of unsettled dust sampled. Particle sizes for settled dust range between 5 to < 10 μm. Eighteen percent of these particles are less than 5 μm in size.

Seventy eight percent of the element in both settled and unsettled dust in this study was carbon. Previous studies have showed that carbon is a effective in inducing cytotoxicity and can lead to inflammatory reaction in the respiratory tract[7]. Whilst Sulphur (S), nitrogen (N), potassium (K), calcium (Ca) and iron (Fe) will cause hypersensitivity in respiratory tract leading to bronchoconstriction of the tract[8].

Table 1: Percentage distributions of elements in unsettled and settled dust

Unsettled	(Mean±SD)	Settled	(Mean±SD)
C	78.71±8.53%	C	78.18±2.64%
O	9.34±3.99%	O	17.75±5.61%
Mg	0.54±0.39%	Mg	0.15±0.08%
Al	5.67±8.63%	Al	0.07±0.07%
Si	2.51±0.95%	Si	0.48±0.42%
S	0.30±0.17%	S	0.10±0.06%
Cl	0.16±0.13%	Cl	0.28±0.22%
K	0.35±0.29%	K	0.20±0.19%
Ca	2.29±2.62%	Ca	0.37±0.27%
Fe	0.41±0.25%	P	0.14±0.14%
Na	0.31±0.25%	Na	0.28±0.09%
Br	0.13±0.14%		

Interestingly, 15.11% of the dust fractions were elongated and oval shaped particles. These kind of particles can easily penetrate into alveoli and lead to respiratory problem. This study did not determine the amount of elongated and oval particles sufficient to cause any respiratory problem, because the amount of particles was not determined that were inhaled in this stable in relation to a diseased state. However, these fractions did not manifest clinical signs that are indicative of a respiratory tract disease.

In this study, using VPSEM-EDX to quantify the particle size, shape and composition was time consuming. This study reports such a use for the first time and can be evaluated against existing conventional methods. It would be interesting to examine and identify particles on respiratory epithelium of horses at post mortem.

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