

Airborne dust and aeroallergen concentration in a horse stable under two different management systems

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Summary

Airborne dust concentration (ADC) was measured in 2 different horse management systems using an Andersen cascade impactor in the box-stall, and a personal Marple cascade impactor attached to the halter to measure ADC in the breathing zone. The levels of aeroallergens implicated in chronic obstructive pulmonary disease were measured by radioallergosorbent-inhibition immunoassay. A conventional management system (System C) utilising hay feed and straw bedding, and a recommended environment (System R) utilising wood shaving bedding and a complete pelleted diet were studied. In the stall, total and respirable ADC (geometric mean) were significantly higher in System C (2.55 mg/m³; 0.44 mg/m³, respectively) than in System R (0.70 mg/m³; 0.20 mg/m³, respectively). In System C, the total and respirable ADC in the breathing zone (17.51 mg/m³; 9.28 mg/m³) were much higher than in the stall, but values in both regions were similar in System R (0.52 mg/m³; 0.30 mg/m³). Major aeroallergens were significantly higher in System C than in System R: *Micropolyspora faeni* (1423 ng/m³ and 705 ng/m³), *Aspergillus fumigatus* (1823 ng/m³ and 748 ng/m³), and mite allergens (1420 ng/m³ and 761 ng/m³). Measurement of ADC with personal samplers indicates that the very high inhalation challenge in the breathing zone is not reflected in measurements of stall air quality. When compared with System C, System R produced only 3% of the respirable dust burden in the breathing zone and a decreased aeroallergen challenge.

Introduction

Airborne dusts and aeroallergens are considered to be major factors in the aetiology of chronic obstructive pulmonary disease (COPD), also known as 'heaves', a condition common in stabled horses. Clinical signs of COPD are intermittent and probably result from a reversible pulmonary hypersensitivity reaction to inhaled barn dusts in susceptible horses (Alexander 1959; Cook and Rosedale 1963; Lowell 1964; Gerber 1973; Halliwell *et al.* 1979; McPherson *et al.* 1979b). Signs regress when horses are removed from the enclosed areas and kept on pasture (Derksen *et*

al. 1987). Airborne dust concentrations have a profound influence on the respiratory health of the horse and increased levels are reported to increase the severity (Clarke *et al.* 1987) and duration (Burrell 1985) of lower respiratory tract inflammation. Management regimens utilising alternative bedding and feed materials are recommended for stabling of COPD-susceptible animals (Thurlbeck and Lowell 1964; Eyre 1972; Thomson and McPherson 1984; Clarke 1987; Grunig *et al.* 1989).

The objectives of this study were to determine whether a change in the management system from a conventional system, in which COPD-susceptible animals develop clinical signs, to a system recommended for the stabling of COPD-susceptible animals would significantly alter the aeroallergen concentration and the level of airborne dust both in the stall and in the pony's breathing zone. Furthermore, to assess the hazard of the airborne dust, it was divided into fractions according to particle sizes that deposit in different regions of the respiratory tract (Lippman 1970). The respirable dust concentration, the portion that reaches the peripheral airways, is considered a good index of the health hazard posed by airborne dust inhalation (Air Sampling Procedures Committee 1984; Lippman 1989).

Materials and methods

Sampling was conducted in a 7 x 12 m (450 m³), 4-stall barn. Two ponies were kept in each stall at all times. The barn was constructed of a steel frame with a concrete block base extending up to 1.5 m and aluminium siding lined with plasterboard. The stalls were divided by wood plank walls, and each stall had a steel bar door. The area had an insulated ceiling and a concrete floor. A mechanical ventilation system consisting of an inlet fan operating at 85 m³/min, and an exhaust fan were the only sources of ventilation. The main entrance to the barn, a 2 m x 2 m metal door, was only opened during stall cleaning and feeding; there was a separate personnel door. Stalls were cleaned out daily in the morning. All soiled or damp bedding was removed and deep litter was avoided. Water and feed were provided *ad libitum*. The feed was added twice daily, in the morning and late afternoon.

Management systems

Conventional management system (System C): Ponies were bedded on straw and fed obviously dusty hay. Daily supplies of

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Fig 1: Airborne dust concentrations within the pony's breathing zone were measured with a 6-stage Marple Personal Cascade Impactor attached to the halter. The Genesis air pump to operate the sampler was attached to a surcingle and operated at 2 litres/min.

hay and straw were stored in front of the stalls. System C has been used to induce signs of COPD in our horses and ponies with a history of heaves.

Recommended management system (System R): Based on recommendations by Thomson and McPherson (1984), Clarke (1987) and Grunig *et al.* (1989), ponies were bedded on wood shavings and fed a complete pelleted diet (Horse chow pellets, Purina Mills, Inc., St. Louis, MO, USA). No materials were stored in the barn.

Each management system was introduced at least 1 week before sampling to establish steady-state airborne dust levels. All animals in the barn were managed in the same manner.

Airborne dust concentration sampling procedures

Sampling of the airborne dust concentrations in the general area of the stall was conducted with a 5-stage cascade impactor (Model 65-800, Anderson Samplers, Inc., Atlanta, GA, USA) mounted on an air pump (Air Sentinel TM; Quan-Tec-Air, Inc., Rochester, MN, USA). The dust sampler was operated at a flow rate of 220 litres/min, on a shelf in the corner of the stall, at an air inlet height of 1.5m. The dust collected on desiccated, pre-weighed fibreglass filters (GMW-65-350, General Metal Works, Inc., Village of Cleves, OH, USA). The very fine dust was collected on a back-up filter of polytetrafluoroethylene (PTFE) membrane (0.3 µm pore size) (Quan-Tec-Air, Inc.).

Dust within the pony's breathing zone was measured with a 6-stage Marple personal cascade impactor (Sierra Instruments Series 290, Sierra Instruments Inc., Carmel Valley, CA, USA) attached to the halter at the angle of the jaw. The pump (Model gn-5, Ametek, Inc., Largo, FL, USA) to operate the sampler was attached to a surcingle belt and operated at 2 litres/min (Fig 1). The pump flow was regularly calibrated (Mini-Buck calibrator, Model M-5, A.P. Buck, Inc., Orlando, FL, USA). The samples were collected on pre-weighed mylar media filters (C-290-MY, Sierra Instruments Inc.) which had been sprayed with a light coating of silicone grease. A back-up PTFE membrane filter (Quan-Tec-Air, Inc.) collected the very fine dust. The aerodynamic diameters of the cut-off levels for each stage of both the personal and area samplers are shown in Table 1.

After sampling for a selected time period, the collection discs were desiccated to constant weight in an incubator, and reweighed to measure the net particulate accumulation.

The sampling periods were selected based on pilot protocols to ensure that an adequate, measurable mass of dust was collected

TABLE 1: Comparison of collection stage cut-off levels for particles of different mass median aerodynamic diameter (µm) collected by Andersen and Marple cascade impactors

	Cascade impactor type		
	Stage	Andersen	Marple
Non-respirable	1	>8.95	>10.00
	2	>4.58	>6.00
	3	>2.78	>3.50
	4	>1.53	>1.55
Respirable	5	----	>0.93
	6	----	>0.52
	Base	<1.53	<0.52

on each stage without exceeding the sampler's capacity and resulting in collection errors due to overload. Each sampling protocol was continued for 7 days.

Sampling was initially conducted during summer, but because of sampling problems, insufficient samples were obtained from the Marple personal impactor in System R and so these studies were repeated during the following winter. The Andersen impactor was not available at that time and 2 Marple impactors were used simultaneously to measure both the breathing zone and stall dust levels.

Comparison of Marple and Andersen sampler collection characteristics

An additional protocol was necessary to attribute any differences in the results obtained from the 2 samplers to a real difference in the environment rather than to differences in the collection efficiencies of samplers. The samplers were placed side by side, ~1 m above the floor and 1 m apart in the centre of the stall that was under System C. Paired dust samples were collected for 6 h on 5 consecutive days by the methods described previously. The total airborne dust concentrations measured by the 2 samplers did not differ. However, the respirable airborne dust concentrations and percentage respirable dust measured by the 2 samplers were significantly different. The latter result was not surprising, because the cut-off point for respirable dust differed (Table 1) and a greater proportion of the total airborne dust was classified as respirable by the Marple sampler.

Aeroallergen concentration sampling procedures

A volumetric air sampler (Air Sentinel TM; Quan-Tec-Air, Inc.) was placed on a shelf in the corner of the stall. Airborne dust particles were collected onto fibrous-backed PTFE membrane filters with a pore size of 0.3 µm (Quan-Tec-Air, Inc.). Samples were collected for 30 mins at a calibrated flow rate of 3 litres/sec. To represent a 24-h period and avoid excessive influence of any daily variations in dust load or composition, the 30-min sampling periods were randomly spread over 5 days. After sampling, the filters were stored in sealed, plastic bags for further analysis at Mayo Clinic Allergy Research Laboratory.

The aeroallergen analysis was by radioallergosorbent inhibition (RAST) immunoassay (Reed 1982; Swanson *et al.* 1985; Campbell *et al.* 1989). The PTFE membrane that contained the dust sample was separated from its fibrous backing and extracted overnight in 2-3 ml 0.1 M-phosphate buffer, 0.2% bovine serum albumin, pH 7.6, and 50% glycerine. The sample was decanted into a Brinkmann vial (Brinkmann Instruments, Westbury, NY, USA) and centrifuged at 10,000 *g* for 5 mins to remove the solid particles. The supernatant was decanted into a clean vial and stored at -20°C until analysed (Campbell *et al.* 1989).

The allergens that were tested were those frequently

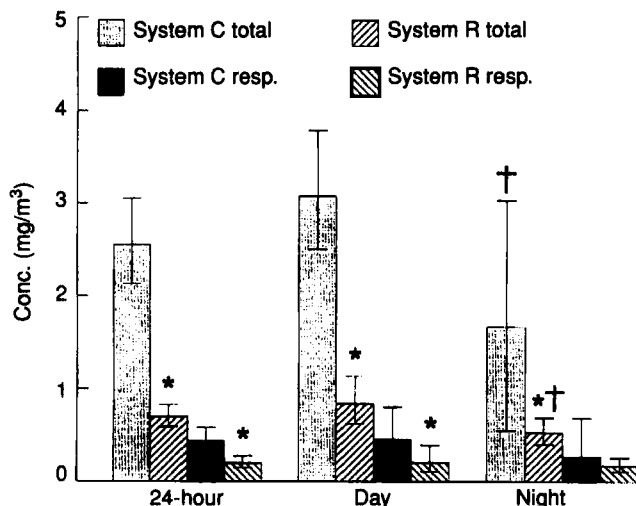


Fig 2: Comparison of the total and respirable (resp) airborne dust concentrations (geometric mean $\pm$ sd) in the horse stables under Systems C and R. * $P < 0.05$ between management systems. † $P < 0.05$ between day and night.

implicated in the aetiopathogenesis of COPD (Eriksen 1986; Mansmann and Strouss 1976; Lawson *et al.* 1979; McPherson *et al.* 1979a; von Hockenjos *et al.* 1981; Zeitler 1984; Sasse *et al.* 1986; Clarke 1987; Campbell *et al.* 1989; Donham *et al.* 1989) and included *Micropolyspora faeni* now renamed *Faenia rectivirgula*, *Aspergillus fumigatus* and *Thermoactinomyces vulgaris* (thermophilic fungi and actinomycetes) and *Lepidoglyphys destructor* (storage mite).

Quantification of the allergens in the air filter extracts was performed by plate-RAST inhibition. The solid-phase reference allergen was prepared as follows: the reference allergens (described by Campbell *et al.* 1989) were coated onto the surface of Immulon-2 microtitre Removawell strips (Dynatech Laboratories Inc., Alexandria, VA, USA). Each well received 100 μ l reference allergen containing 1.0 μ g protein in sodium bicarbonate buffer (200 mmol/l) titrated with 200 mmol sodium carbonate/litre to pH 9.2. The plates were incubated at room temperature (22°C) in a humid box and washed 3 times with RAST wash buffer (0.1 mol/litre phosphate buffer, 1% Tween 20) using a Nunc Immuno-wash 12 plate washer (Nunc-Inter Med, Copenhagen, Denmark). A standard inhibition curve was generated with a reference allergen standard.

Samples (50 μ l) of the test allergen at various concentrations were placed in the allergen coated microtitre wells with 50 μ l specific human antisera (Campbell *et al.* 1989). The plates were incubated at room temperature overnight in a humid box, washed 5 times, and 100 μ l (20 ng) ^{125} I-labelled antihuman IgE or protein A for IgG antibodies were added to each well. The plate was incubated again at room temperature in a humid box for 18 h. The plate was subsequently washed 5 times, and the Removawell (Dynatech Laboratories, Inc.) strips were broken apart into 16 x 125 mm glass test tubes and counted in a gamma scintillation counter.

Statistical analysis

Because airborne dust concentrations are not normally distributed (Ayer 1989), the data were expressed as a geometric mean and non-parametric statistics were applied. The Mann-Whitney U test was used to compare concentrations in the 2 systems and the signed rank test compared the simultaneously measured airborne dust concentrations in the stall and breathing zone. Aeroallergen concentrations under the 2 management systems were compared by Student's *t* test. The confidence limits were set at 95%.

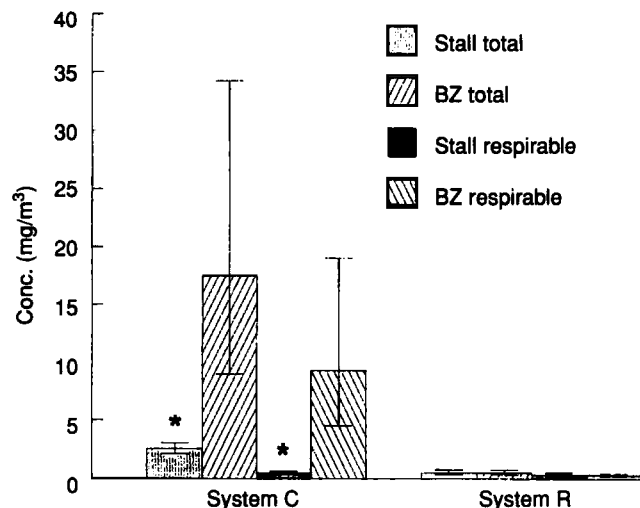


Fig 3: Total and respirable airborne dust concentrations (geometric mean $\pm$ sd) in the stall and in the pony's breathing zone (BZ) under Systems C and R. * $P < 0.05$ compared with BZ values in this system.

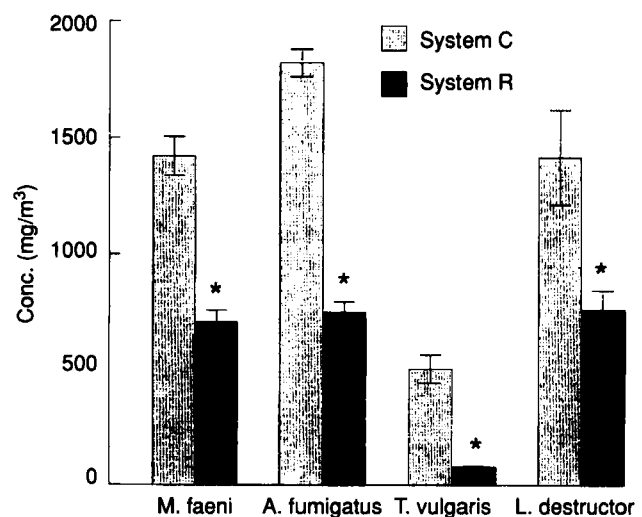


Fig 4: Aeroallergen concentrations (mean $\pm$ se) in the stall under Systems C and R. * $P < 0.05$ between management systems.

Results

Effect of changes in management on the airborne dust concentrations in the stall

The change from System C to System R more than halved the total airborne dust concentrations in the stall (Fig 2). In both systems, the mean total airborne dust concentration was significantly higher during the daytime period (07:00–19:00 h), than during the period of 19:00–07:00 h. The total airborne dust concentrations measured at night were 62.6% and 54.3% of the daytime values for System R and C, respectively.

The concentrations of respirable airborne dust in the stall measured over 24 h and during the daytime period were significantly higher in System C than in System R (Fig 2). In System R, the 24-h respirable airborne dust concentration was 46% of that measured in System C. The nighttime respirable dust concentrations did not differ significantly between the 2 systems. Despite the large, statistically significant diurnal variations in the

total dust levels measured in both systems, there were no significant diurnal changes in the levels of respirable dust in either system. Respirable dust formed a larger percentage of total dust in System R (29%) than in System C (17%).

Effect of changes in management on the airborne dust concentrations in the pony's breathing zone

The total and respirable airborne dust concentrations in the breathing zone under System R were each only 3% of the concentrations measured under System C (Fig 3). Respirable dust formed about half of the total dust in both systems. In System C, the total and respirable airborne dust concentrations were significantly higher during the day than at night, but the percentage of dust that was respirable did not alter (data not shown). Owing to the low airborne dust concentration in the breathing zone with System R and the low flow rate of the Marple sampler, it was not possible to collect enough dust in 12 h to distinguish differences in daytime and nighttime dust concentrations under System R.

Comparison of airborne dust concentrations in the stall under System R in summer and winter

With System R, there was no significant difference between the total airborne dust concentrations in the stall measured during the summer using an Andersen cascade impactor, and those measured during the winter with a Marple cascade impactor. After allowing for the differences in cut-off values for respirable dust in the 2 samplers, there was no significant difference in either the stall concentrations of respirable dust or in the percentage of respirable dust measured in System R in summer and in winter.

Comparison of airborne dust concentrations in the stall and breathing zone under the two systems

Under System R, the total and respirable airborne dust concentrations in the stall and breathing zone did not differ significantly (Fig 3). In contrast, the mean 24 h total and respirable airborne dust concentrations in the pony's breathing zone under System C were significantly higher, by factors of 7 and 21 respectively, than the simultaneously measured stall levels (Fig 3). Although there was no difference in the day and night levels of respirable dust measured in the stall under System C, the breathing zone respirable dust levels decreased by half (13.1 mg/m³ during the day to 5.25 mg/m³ at night).

Comparison of the aeroallergen concentrations in the stall under Systems C and R

The mean concentrations of aeroallergens were significantly higher

in System C than in System R (Fig 4). Under each system, the concentrations of *M. faeni*, *A. fumigatus* and *L. destructor* aeroallergens were similar to each other. *T. vulgaris* levels were lower than those of the other aeroallergens.

Discussion

The study demonstrated that System R, which utilised a pelleted feed and woodshaving bedding, produced lower airborne dust and aeroallergen concentrations than did System C, which utilised hay feed and straw bedding. There are 3 main methods of decreasing the airborne dust concentration in a building. The first, decreasing contaminant release by modifying the source materials, was employed in this study. The inhalation challenge of airborne dust and aeroallergens was decreased by changing the bedding and feed materials. The second method decreases the rate of particle release by decreasing the degree of agitation of the source materials: eating relatively dust-free pellets releases fewer dust particles than when eating hay. The third method is to increase the particle clearance rates by increased ventilation that removes air with its suspended dust load from the building. This is the most effective method to clear fungal and actinomycete spores, which sediment slowly due to their small aerodynamic diameter (Clarke 1987). The building ventilation rates were kept constant in this study, and all variables, other than the bedding and feed materials, were controlled.

The mean total airborne dust concentration in System C (2.55 mg/m³) was generally higher than previously measured concentrations of 0.41 mg/m³ (Crichlow *et al.* 1980), 0.55–3.63 mg/m³ (Sasse *et al.* 1986), and 0.63 mg/m³ (Zeitler 1985). However, under System R the value (0.53 mg/m³) was similar to those reported previously. The higher dust concentration under System C in the present study could be due to the use of a notably dusty hay and the positioning of the sampler within the stall rather than in adjacent corridors. All these values are lower than the threshold limiting value of 10 mg/m³ for nuisance particulates established for human exposure over an 8-h period (American Conference of Governmental Industrial Hygienists 1977). However, this limiting value applies to inert, insoluble, airborne dusts, and not to airborne particulates that act as allergens. Furthermore, horses may be exposed continuously to high dust levels if they are kept indoors for 24-h periods.

Under both management systems and in earlier investigations (Crichlow *et al.* 1980; Zeitler 1985; Clarke 1987), the total airborne dust concentrations were higher during the day than at night. This would be expected because of greater personnel activity during the day. Although the total dust concentration decreased during the night under both management systems, the respirable dust concentration did not. This observation tends to agree with that of Zeitler (1985) and suggest that the factors that decrease the airborne dust concentration at night are size-selective. Presumably, the larger

TABLE 2: Calculated aeroallergen inhalation challenge in the stall and breathing zone under Systems C and R

	<i>M. faeni</i> System		<i>A. fumigatus</i> System		<i>L. destructor</i> System		<i>T. vulgaris</i> System	
	C	R	C	R	C	R	C	R
Aeroallergen conc. (ng/m ³)	1423	705	1823	748	1420	761	502	79
Aeroallergen inhalation challenge (mg/day) ^a	0.123	0.061	0.158	0.065	0.123	0.066	0.043	0.0072
Aeroallergen challenge in total dust of breathing zone (mg/day) ^b	0.845	0.060	1.085	0.064	0.845	0.065	0.295	0.0071
Aeroallergen challenge in respirable dust of breathing (mg/day) ^c	2.594	0.091	3.332	0.097	2.594	0.098	0.907	0.110

The inhalation challenge values were calculated for a horse with a mean minute ventilation of 60 litres/min.

^a Calculated using aeroallergen concentration x horses daily ventilation.

^b Calculated using the ratio of the total airborne dust concentrations in the breathing zone and in the stall.

^c Calculated using the ratio of the respirable airborne dust concentrations in the breathing zone and in the stall.

particles tend to gravitate out of the air during the night. However, in the Zeitler study, the day-to-night changes in the percentage of dust that was of respirable size (30% during the day and 90% during the night) were much greater than in the present study (System C: 15% during the day and 16% during the night; System R: 24% during the day and 32% during the night). This difference between the two studies is probably due to the more than adequate ventilation rates in the barn used in the present study.

The impact of changing source materials on the dust levels around the head was demonstrated by the differences in both the total and the respirable airborne dust concentrations in the breathing zone when System C was compared with System R (Fig 3). The dust levels in the region of the ponies' nostrils when eating pellets and on wood shaving bedding were only 3% of those present when eating hay and bedded on straw. The instantaneous increase in airborne dust concentrations that occurs when the pony agitates dusty hay during feeding is more likely to be measured by a personal sampler placed close to the nostril than by a stationary stall sampler placed away from the dust source.

When comparing the total and respirable airborne dust concentrations in the breathing zone with those measured simultaneously in the stall, the levels were significantly higher in the region of the horse's nostrils than in the stall only under System C. This difference in the two regions is due to: (1) release of particles close to the personal sampler, (2) a dilution effect as the airborne dust disperses from the source, and (3) the removal of dust-loaded air by ventilation. The present study clearly showed that when the building ventilation rates are high, ~5 air changes/h, the airborne dust concentration measured in the stall grossly underestimated the breathing zone airborne dust challenge. Because ventilation does not remove the dust challenge from the pony's nostrils as it feeds on hay, changing the source material is the only way to decrease dust levels in the breathing zone. This is confirmed by the observation that the breathing zone and stall levels of total and respirable airborne dust did not differ under System R. Few horse housing units have adequate ventilation (Webster *et al.* 1987; Jones *et al.* 1987), especially during the cold months when buildings tend to be closed up to decrease heat loss. A low airborne dust concentration in the horse's breathing zone should therefore be the aim of management alterations.

The reduction in the levels of aeroallergens in the stall paralleled the reduction in airborne dust levels when the management systems were changed. The concentration of airborne aeroallergens under System C was about double that in System R. Unlike respiratory infections, in which the viability and infectivity of the causative organism is important, the incidence and severity of COPD are more closely related to the quantity of allergenic material inhaled and the sensitivity of the individual horse to the specific allergen (Webster *et al.* 1987). System C posed a significantly higher inhalation challenge than did System R for those aeroallergens measured. The levels of these aeroallergens, even the lower levels measured in System R, are well within the range that can cause asthma in sensitised human subjects (C. E. Reed, personal communication).

Aeroallergen concentrations in horse barns have been measured previously using indirect methods that rely on microbial viability or particle identification (Zeitler 1984; Clarke *et al.* 1987). The culture analysis method underestimates the spore prevalence (Burge *et al.* 1977); dust mites that feed on fungal spore will decrease the viability, but not the antigenicity of the spores (von Hockenjos *et al.* 1981; Clarke 1986). An immunochemical technique has the advantage of measuring aeroallergens without regard to the particle viability or defined form, e.g. animal dander (Brown *et al.* 1987) or arthropods (Swanson *et al.* 1985). Each immunoassay was specific for the allergen that was tested. Some cross-reactivity may exist among various species of the same allergen, but no cross-reactivity exists between the 4 allergens tested (M. C. Swanson, personal communication).

Micropolyspora faeni (McPherson *et al.* 1979a), *Aspergillus*

fumigatus (Lawson *et al.* 1979) and *Thermoactinomyces vulgaris* (Lawson *et al.* 1979; McPherson *et al.* 1979a) are considered important in the aetiology of COPD in horses. Their spores are of respirable size aerodynamic diameter 1.91 µm for *M. faeni*, 3.11 µm for *A. fumigatus* and 0.58 µm for *T. vulgaris* (Lacey and Dutkiewicz 1976) and therefore have the potential to reach the peripheral airways. Mites and their faeces also are involved in the development of asthmatic signs in humans (Platts-Mills 1992) and are suspected in the development of COPD in horses (von Hockenjos *et al.* 1981). Low levels of *T. vulgaris* were measured in the present study, in contrast to that of Clarke *et al.* (1987) in which this actinomycete was the most prevalent spore in horse stables. *T. vulgaris* is found on degrading wood chips and might have been more frequent in the present study if a deep litter system had been allowed to develop.

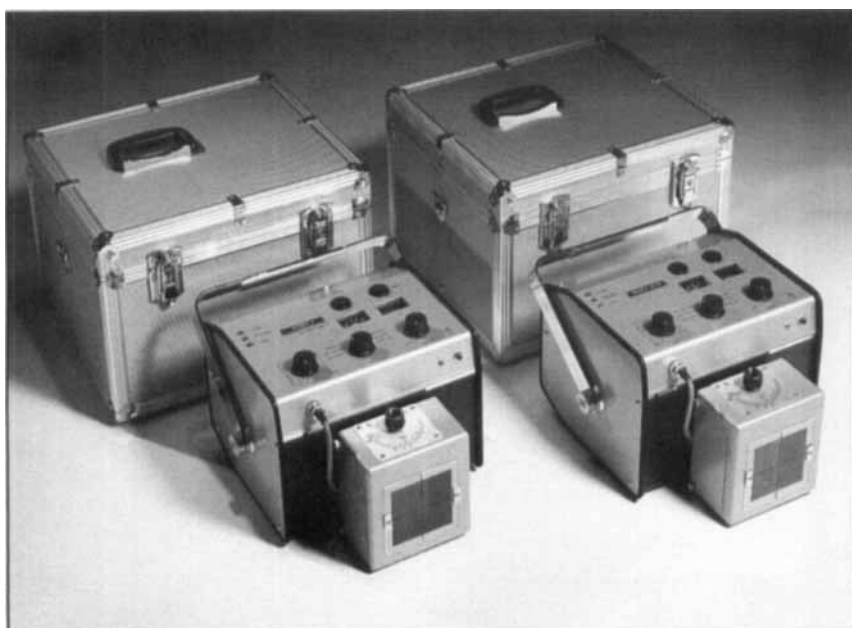
Airborne allergen concentrations were considered in terms of the inhalation challenge dose posed by the 2 management systems (Table 2). Although the aeroallergen concentration was measured in the stall, the challenge dose is likely to be higher in the breathing zone, because the relationship of the airborne dust in the stall and the breathing zone almost certainly also holds true for the aeroallergen levels in these 2 regions. In addition, aeroallergens are of a size that would be in the respirable fraction.

The differences between the 2 management systems became even more apparent when the calculated inhalation aeroallergen challenge doses in the breathing zone were compared. The potential aeroallergen challenge in the breathing zone was 16 times greater under System C than System R, calculated from the ratio of the total airborne dust concentrations, and 30 times higher under System C when the values were calculated using the ratios of the respirable dust concentrations in the 2 regions. These differences in the inhalation challenges posed by the 2 management systems serve to emphasise the importance of utilising materials with a low dust-producing potential to produce a low-challenge environment.

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