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Clinico-Pathological Effects of Atmospheric Ammonia Exposure on Horses

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To clarify the effects of ammonia on the respiratory tract during trailer transports, horses in stalls were exposed to either 2–17 ppm (horse No. 1), which is a level similar to the interior vehicle environment, or 40–130 ppm (horse No. 2) of gaseous ammonia for 40 hr. Clinically, coughing and hypersecretion of nasal discharge, but no significant hematological changes, were observed in the exposed horses. Morphologically, swelling of the tip of cilia in the tracheal epithelium was observed in horse No. 1. In contrast, marked changes in the form of lost cilia and degenerative cytoplasmic change of the tracheal epithelium were observed in horse No. 2. These histological changes suggest a direct effect of ammonia gas on the respiratory tract.

Key words: *airway, ammonia, horse, transport*

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The pathogenesis of equine respiratory disease associated with transport appears to involve a number of intrinsic and extrinsic factors; in particular, it is assumed that adverse environmental conditions during transport, such as dry air, as well as high concentrations of atmospheric ammonia and dust may serve as some of the inciting factors. Leadon *et al.* suggested that a large number of airborne pathogens present in the transport vehicle during transportation may be inhaled, thereby inducing equine respiratory disease [4]. They also have reported the possibility that the desiccating effects of exposure to dry air while the vehicle is in motion may result in diminished mucosal clearance mechanisms in the airways.

In our previous study, we noted that the concentration of ammonia gas components emitted from the buildup of excreta in the vehicle increased in proportion to the duration of transport [6]. We could not, however, elucidate whether this increased exposure of ammonia gas adversely affected the mucous lining or not.

The objective of this study, therefore, was to elucidate the effects of ammonia gas as the inducing factor in equine respiratory disease by exposing horses to two different concentrations of ammonia gas in their stalls and examining clinical and histopathological changes in the respiratory tract.

Studies were carried out on three healthy female thoroughbred horses, with a age of 4 years old, which were free from respiratory diseases. Out of them, two were exposed to ammonia gas; one horse (horse No. 1) was at the level of 2–17 ppm of ammonia gas (stall A), which is the same range of concentration as in a trailer during transport [6], the other horse (horse No. 2) was at 40–130 ppm (stall B), which is the maximum allowable exposure concentration for humans [1]. The third horse (horse No. 3) was served as the control without any exposure to ammonia gas (stall C). The exposure time was 40 hours which is almost equal to the time required for transport between the Hokkaido district and Ritto training center (distance, 1708 kilometers). During the exposure, all horses were given free access to hay and water. The experimental set-up for the exposure was shown as in Fig. 1, where ammonia gas was mixed

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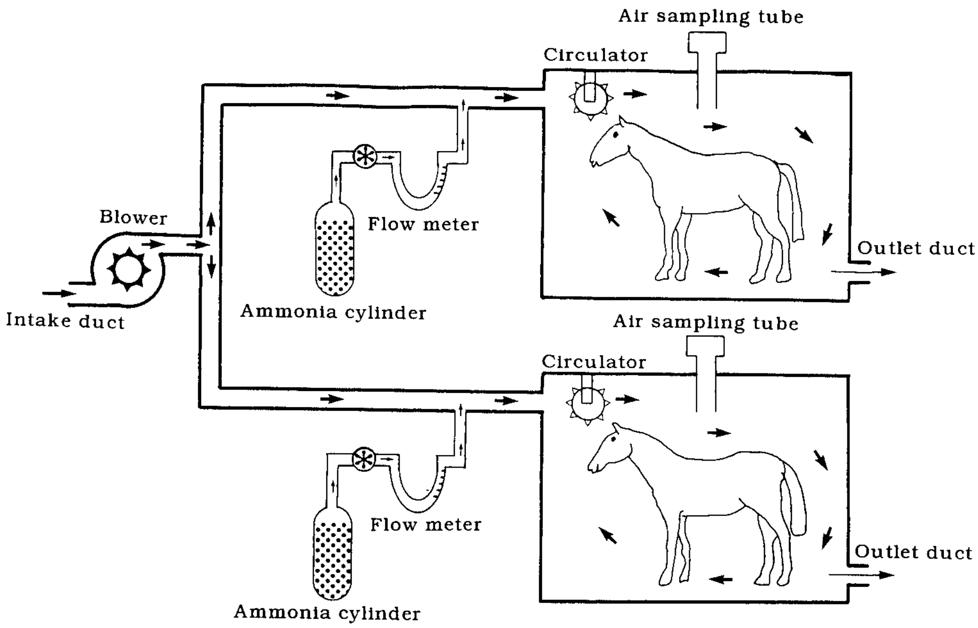

Fig. 1. Ammonia exposure apparatus.

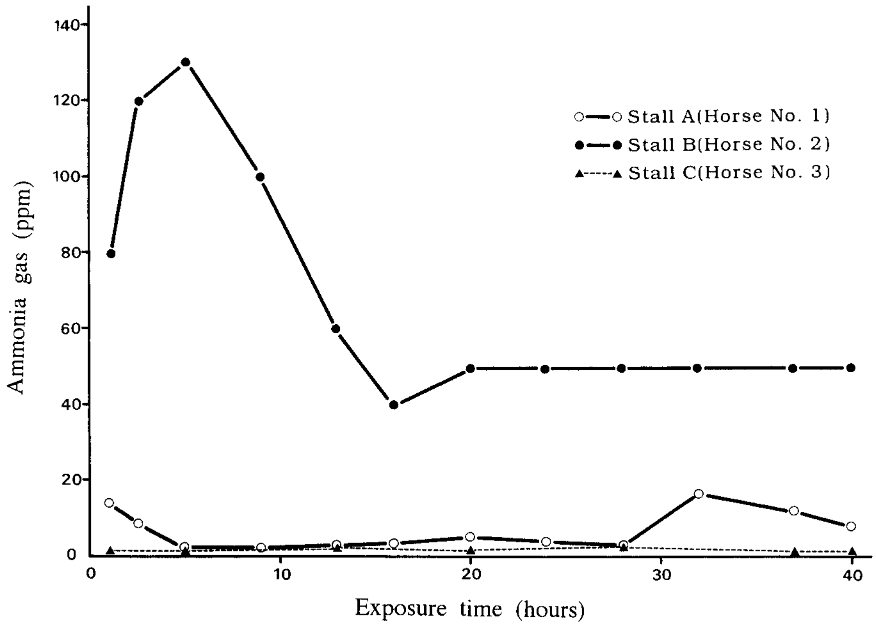

Fig. 2. Exposed ammonia concentration in stalls.

with fresh air and introduced continuously into a sealed stall at the rate of 100 l per minute. The mixed gas introduced into the stalls was agitated by a circulator and removed by an exhaust vent. The ammonia gas concentration was exactly analyzed twelve times during the 40 hr exposure using the methods described previously [6]. The concentration of ammonia gas was maintained at the level within the desired range described above by adjusting the valve of an ammonia cylinder.

The following clinical examinations were made for all horses during exposures : rectal temperature, heart rate, respiratory rate, visible mucous membrane color, gut motility, vesicular breathing, coughing and nasal discharge. Hematological data before and after exposure included the packed cell volume and the number of total red blood cell, total leukocyte, eosinophil, neutrophil and lymphocyte. In addition, histological examinations with light microscopy, scanning and transmission elecrton microscopy were performed on the tracheal biopsy specimens obtained before and after the exposure period.

During the experimental period, the temperature in the stalls was ranged from 9.7 to 15.4°C, with relative humidity from 82.6 to 96.4%. The ammonia concentration was 2–17 ppm in stall A, 40–130 ppm in stall B, and 0.2–1.9 ppm in stall C (Fig. 2).

Horses 1 and 2 exhibited hypersecretion of nasal discharge. The severity was more marked in horse No. 2. No significant changes in rectal temperature, heart rate, respiratory rate, visible mucous membranes, enterocinesia or breathing murmur were observed for the three horses.

The number of erythrocytes, leukocytes, neutrophil, lymphocytes and also packed cell volume in the two exposed horses decreased significantly. The number of eosinophils decreased in horse No. 1, but were unchanged in horses No. 2 and No. 3 (Table 1). It is known that the decrease of peripheral cell counts are caused by hemorrhage, hemolysis and sequestration in the spleen etc. Since the hemorrhage and hemolysis were not observed in these cases and the sequestration in the spleen was not examined, the cause of these hematological changes could not be determined.

By the light microscopic examination, no significant changes were observed in the pre-exposure specimens for any of the horses. At the time of post-exposure, swelling and irregular distribution of tracheal epithelium and edema of the submucosa were observed in horse No. 2. The secretory granules within goblet cells and tracheal gland cells were markedly decreased. In the horse No. 1, a slight swelling of the cilia tips was observed at post-exposure by scanning electron microscopy. In the horse No. 2, a loss of cilia and swelling of the cilia tips was more frequent, and the surface of the cilia was rough with an irregular pattern of arrangement (Fig. 3). By the transmission electron microscopic examination, the basal bodies of the ciliated cells in horse No. 1 were observed to have a zonally electron dense area following the ammonia exposure. In horse No. 2, the cytoplasm of ciliated cells frequently demonstrated an increased electron density. The crista of the mitochondria was irregular and enlarged. The number of free ribosomes was decreased, vacuoles possibly originated from the smooth endoplasmic reticulum were increased in the cytoplasm, and the intercellular spaces were distended. There was a decrease in the number of cilia, and the microtubles in the basal body area were indistinct or absent all together. On occasion, the unit membrane of the cilia showed a cystic enlargement (Fig. 4). The mucous droplets of the tracheal gland cells were swollen, demonstrating a low electron density (Fig. 5). In the non-exposed horse No 3, no significant ultrastructural changes were found.

Although significant clinical and histological changes were observed in exposed horses, hematological abnormalitis were not seen in the blood samples. This suggests that ammonia gas has a

Table 1. Hematological changes

	Horse No. 1		Horse No. 2		Horse No. 3	
	Pre-[a]	Post-[b]	Pre-[a]	Post-[b]	Pre-[a]	Post-[b]
Erythrocyte ($\times 10^4$/mm^3)	880	555	1020	665	850	720
PCV[c] (%)	43	27	50	31	48	45
Leukocyte (/mm^3)	7200	4200	9600	6600	8100	7300
Eosinophil (/mm^3)	67	34	102	114	98	80
Neutrophil (/mm^3)	4400	2400	4100	3200	4500	4300
Lymphocyte (/mm^3)	2700	1600	5400	3200	3500	3000

Remarks. Pre-[a]: Pre-exposure. Post-[b]: Post-exposure. PCV[c]: Packed cell volume.

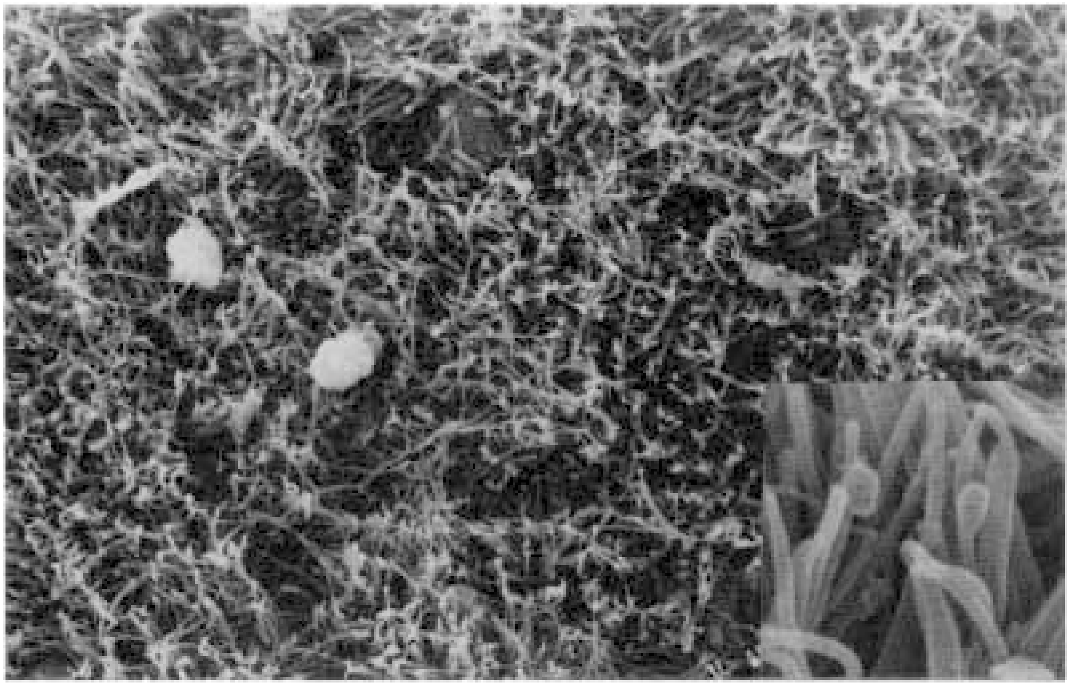

Fig. 3. Scannig electron microscopy findings of horse No. 2. Surfaces of the cilia were rough, with an irregular pattern of distribution at post-exposure. × 2,500. Inset: Swelling of the cilia tips. × 25,000.

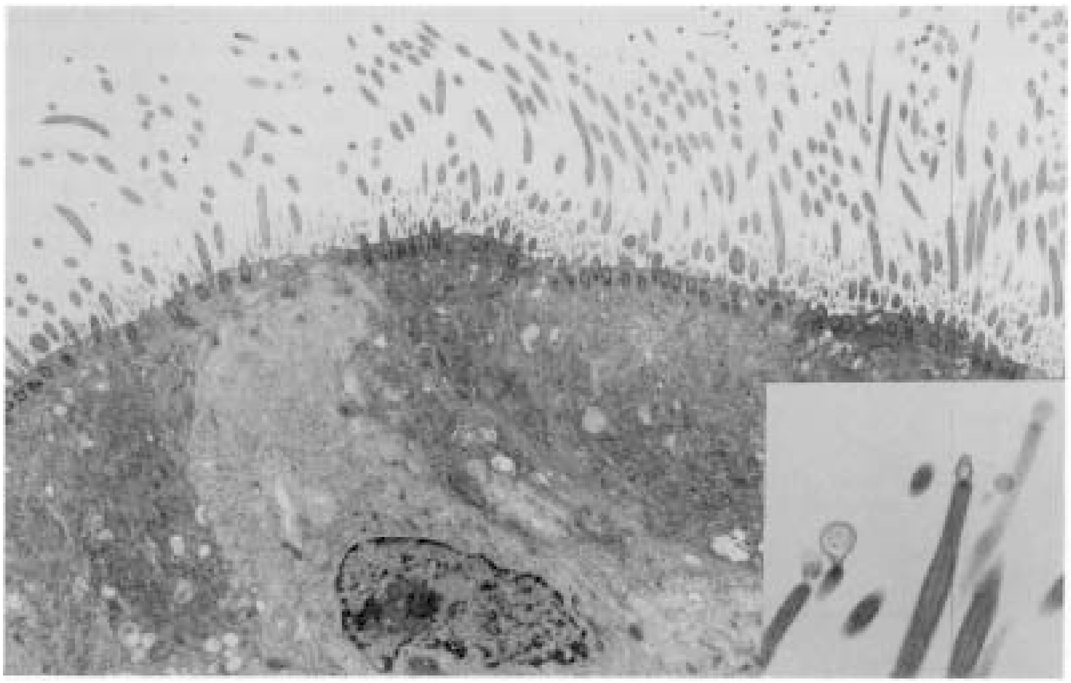

Fig. 4. Transmission electron microscopy findings of horse No. 2. Cytoplasm of ciliated cells showed increased electron density. The number of free ribosomes was decreased, vacuoles possibly oriented from smooth endoplasmic reticulum were increased in the cytoplasm. The number of cilia was decreased. × 5,000. Inset: The unit membrane of the respiratory cilia showed cystic enlargement. × 15,000.

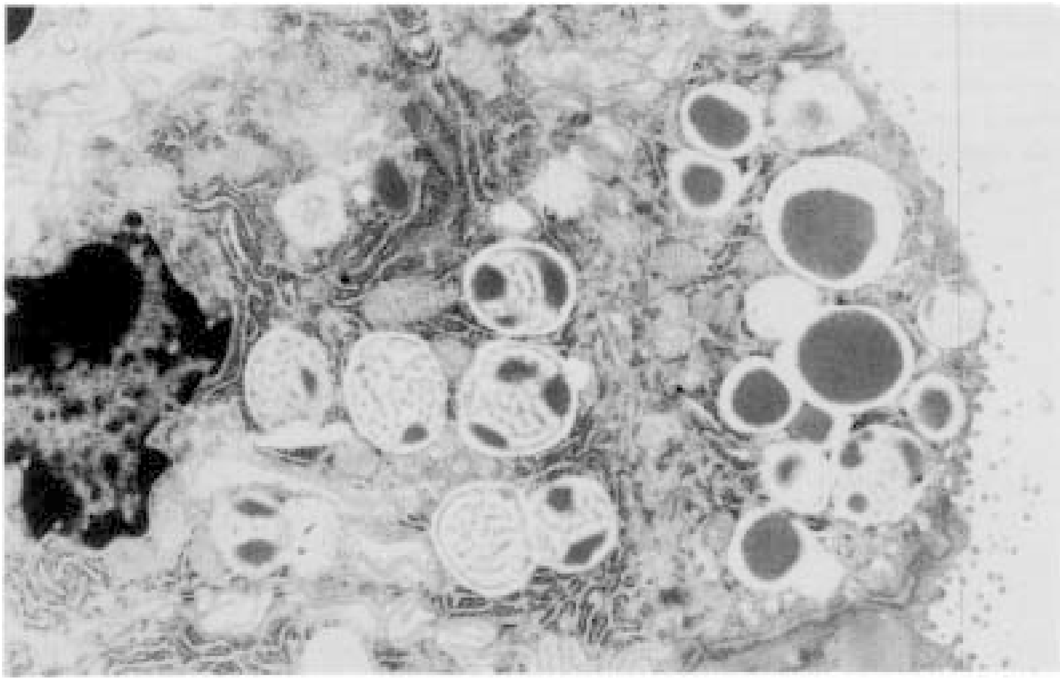

Fig. 5. Transmission electron microscopy findings of horse No. 2. The mucous droplets of the tracheal gland cells were swollen, showing low electron density. × 13,000.

divert deleterious effect on the respiratory tract rather than a systemically-mediated effect. A clear distinction was noted in the severity of the clinical and morphological changes for the two exposures. This suggests that the degree of damage in the respiratory tract is associated with the concentration of ammonia. Histopathological examination of horse No. 1 indicated a slight change in the swelling of the cilia tips and high electron density of the cell borders in ciliated cells. In contrast, in horse No. 2, there was a loss of cilia and the degenerative cytoplasmic change was evident in ciliated cells. The lower electron density of the mucous droplets and the enlargement of the rough endoplasmic reticulum, disappearance of microtubles, high electron density of cellular borders, and cystic enlargement of cilia observed in the present study closely resembled the pathological effects following the inhalation to noxious gases such as sulfur dioxide [2, 5]. These findings suggest that ammonia gas has a direct effect on the exposed cellular surfaces and cilia of the respiratory tract [3] and that this noxious gas component can be considered as one of underlying factors in the elicitation of respiratory disorders associated with the transport.

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