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Pathology of Equine Respiratory Disease Occurring in Association with Transport

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Summary

Eight young thoroughbred horses, taken 1858 km by road (travelling time, 41 h), were examined to assess the pathological nature of respiratory disease associated with transport. Three of the horses showed clinical abnormalities including pyrexia, coughing, leucocytosis and neutrophilia after the first 20 h of transportation. Endoscopical examination of the trachea revealed exacerbation of airway inflammation as a result of transport in two of the three affected horses. A consistent finding in the affected horses was focal serous neutrophilic pneumonia affecting the cranio-ventral portion of the caudal lung lobe with a propensity to affect the right lung. *Streptococcus equi* subspecies *zooepidemicus* was isolated from the pneumonic areas, in which corresponding bacterial antigens were identified immunohistochemically. Viral cultures from the pneumonic lesions proved negative for respiratory viruses. It is suggested that transport predisposes the upper respiratory tract and the lower airways to invasion by the bacterium, with episodic pyrexia and acute pneumonia.

Introduction

Transportation is believed to play an important role in equine respiratory infections. In one study, 24·4% of horses with pneumonia had recently been transported more than 500 miles (Raphael and Beech, 1982). In a later study, in the UK, eight of 11 affected horses had been subjected to long-distance travel within the previous 24 h (Mair and Lane, 1989). Pulmonary defence mechanisms may be adversely affected by stress (Bayly *et al.*, 1986; Chrisman, 1987; Traub-Dargatz *et al.*, 1988) and airborne pathogens or irritants may be inhaled (Chrisman, 1987; Leadon *et al.*, 1990) during transport. Further studies on the effects of transport on equine respiratory infections have been made by Mair and Lane (1989), Rooney (1991), Hayakawa *et al.* (1993) and Oikawa *et al.* (1994), but the pathogenesis is still obscure.

The purpose of the present study was to study the pathological events occurring in the respiratory tract of horses developing acute respiratory disease during transport.

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Materials and Methods

Experiment 1

A pilot experiment was carried out to clarify the clinical effects of transit-related respiratory disease.

Horses. In April 1993, 29 thoroughbred horses aged 23 to 27 months (18 male, 11 female), without any previous history of clinical respiratory disease, were transported 1708 km by road, a journey taking approximately 36 h. Throughout the journey, after each period of 4–5 h the horses were rested for 0·5–1 h. Commercial trucks (six-horse capacity) were used, four or five animals being loaded into each truck. The horses had ready access to hay throughout the journey and were given water during each rest period. During and after transportation, the horses were treated in accordance with the guidelines for the humane use of experimental animals laid down by the Equine Research Institute, Japan Racing Association.

Clinical examination. The horses were observed for clinical signs of disease. Rectal temperatures were recorded 4-hourly during transport. Blood samples were taken from the jugular vein of each horse before departure and on arrival. Total leucocyte counts were determined by an automated counter, and differential counts were obtained by examination of May-Grünwald-stained blood smears.

The criteria used to define transit-related respiratory disease were a rectal temperature of >38·6°C, coughing and a nasal discharge, and lethargy during transport. Only horses with a rectal temperature in excess of 38·6°C were considered to be affected, regardless of whether the other criteria were present or absent.

Serological examination. Serum samples collected from the horses on days 30, 7, and 0 before departure and days 1, 7, and 30 after arrival were examined for evidence of equine herpesvirus type 1 and equine adenovirus by the complement fixation test, and of equine rhinovirus type 1 and calf diarrhoeal coronavirus by the serum neutralization test. The latter infection is a frequent cause of pyrexia in young horses in Japan. Equine influenza virus and equine viral arteritis virus were not present in the Japanese horse population during the period of this experiment.

Experiment 2

This was conducted in August 1993 to study the pathology of acute respiratory disease of horses in transit.

Horses. The eight thoroughbred horses used, aged 27 to 29 months (two male, six female), were in good health as ascertained by clinical examination (Table 1).

Transportation. The horses were loaded four to a truck, each truck being designed to carry six horses separated by partitions. These horse boxes were draughty. The animals were taken 1858 km by road, the total travelling time being 41 h. Throughout the journey, the horses were rested for 1 h after each 5-h period of transport. The horses had free access to hay throughout the journey and were offered water during the rest periods.

Vehicle interior environment. The ambient temperature, relative humidity, and aerial dust and ammonia concentrations were recorded at 5- to 7-h intervals throughout the journey, while the vehicle was moving. The air temperature and relative humidity were recorded simultaneously with an instrument that measured these parameters automatically (Climomaster Model 6511; Kanomax Co., Japan). The sampling devices of this instrument were placed centrally in the vehicle 1·0 to 1·5 m above the floor, that is, at the level of the horses' nostrils. The number of airborne particles at the

Table 1
Horses examined

Horse no.	*Sex*	*Age (months)*	*Peak rectal temperature (°C) during journey*	*MP (0–3)*	
				P	*Po*
1	M	28	38·3	1	2
2	M	28	39·2	3	3
3	F	28	39·9	2	3
4	F	27	38·2	1	1
5	F	27	38·2	1	2
6	F	28	39·2	2	3
7	F	29	38·4	0	1
8	F	29	38·2	0	0

M, male; F, female; MP, amount of mucopus visible in the trachea, scored from 0 to 3, as described by Burrell (1985); P, prior to transportation; Po, post-transportation.

same site was measured with a Digital Dust Indicator (Model P-5; Shibata Co., Japan). To assess the atmospheric ammonia concentrations, samples of air were collected from a central site in a gas sampling bottle (20 min at a suction rate of 1 l/min). After sampling, the amount of ammonia in the air was estimated by the indophenol absorptiometric method, that is, sodium phenol-pentacyanonitrosylferrate and sodium hypochlorite solutions were added to the sampling bottle and the absorbance of indophenol blue generated by the reaction with ammonium ions was measured.

Clinical examination. Rectal temperature, depression, coughing, and nasal and ocular discharge were recorded throughout the journey. Total leucocyte count and differential counts were determined for each horse every 5 h during the journey, by the methods described for Experiment 1. Endoscopical examinations were performed before departure and on arrival, by a method described previously (Burrell, 1985).

Pathological examination. Immediately after arrival and on completion of the clinical examination, the horses were killed by intravenously administered sodium pentobarbital, and necropsied. Samples for histological, electron microscopical and bacteriological examination were collected within approximately 1 h of euthanasia.

Histology. After macroscopical inspection, tissues from major visceral organs were sampled and preserved in 10% buffered formol saline for routine processing. Tissue samples from the trachea and lungs were taken from 16 areas (Fig. 1) by a sampling site method described by Blunden and Mackintosh (1991) and from areas with macroscopical lesions. Sections 5 μm thick were cut and then stained with haematoxylin and eosin (HE), Mallory's phosphotungstic acid-haematoxylin (PTAH), periodic acid-Schiff (PAS) and Gram stain.

Electron microscopy (EM). For transmission electron microscopy, representative samples from the trachea and lungs, including macroscopical lesions, were pre-fixed in a solution of glutaraldehyde 2% in 0·2 M sodium cacodylate buffer (pH 7·4) at 4°C for 1 to 2 h, rinsed in 0·2 M cacodylate buffer at 4°C for 2 h, post-fixed in 1% osmium tetroxide, dehydrated in a graded ethanol series, and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate, and viewed with a Hitachi H-600 transmission electron microscope. For scanning electron microscopy, small tissue specimens were pre-fixed in a solution of glutaraldehyde 2% in 0·2 M sodium cacodylate buffer and rinsed in 0·2 M cacodylate buffer. Then they were dehydrated in a graded acetone series and transferred to amyl acetate. The specimens were dried

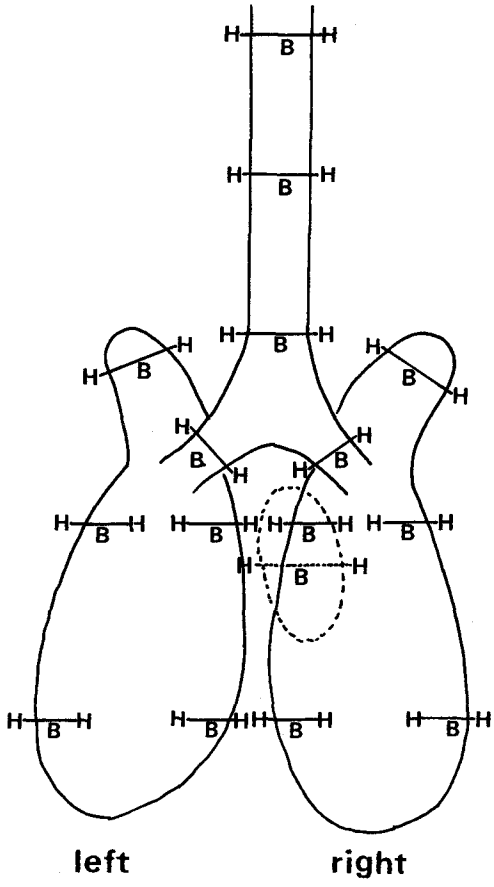


Fig. 1. Sites from which tissue and bacterial samples were taken (dorsal view of lung). B, Site for bacteriological sampling; H, site for histological sampling.

in a critical-point drying apparatus with a CO_2 gas phase, coated with gold, and examined by a Hitachi S-405 electron microscope.

Immunoperoxidase technique. Tissues from pneumonic lesions were fixed and processed by a simplified tissue processing method for immunohistochemical examination as described previously (Oikawa *et al.*, 1994) and then embedded in paraffin wax at 58–60°C. Thin sections were treated immunohistochemically by the avidin-biotin complex immunoperoxidase technique (ABCIT) (Oikawa *et al.*, 1994). Primary antisera against formolized whole *Streptococcus equi* subspecies *zooepidemicus* (*S.z.*) organisms were prepared and used as described previously (Oikawa *et al.*, 1994).

Bacteriological examination. Specimens from major visceral organs including different regions of the trachea and lungs with macroscopical lesions (Fig. 1) were collected aseptically and chopped with sterilized scissors. Pieces of tissue were emulsified and diluted decimally (up to 1 in 10^9) with phosphate-buffered saline. Each dilution (0·1 ml) was spread on a heart infusion agar plate containing horse blood 8%. The plates were incubated aerobically under 10% CO_2 at 37°C for 48 h. The methods for counting organisms in tissues and fluids and for the identification of *S.z.* and other bacteria were as described previously (Oikawa *et al.*, 1994).

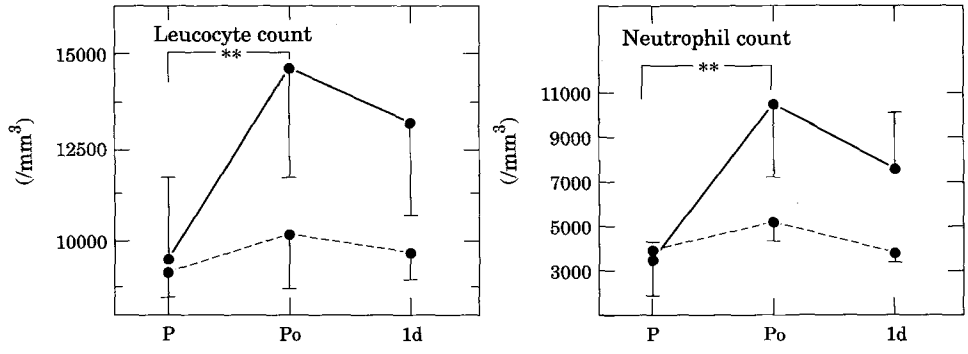


Fig. 2. Changes in white blood cell counts in response to transportation. P, Prior to transportation; Po, post-transportation; 1d, 1 day after transportation; ●—●, horses with pyrexia ($n=13$); ●---●, horses without pyrexia ($n=16$). Values are expressed as the mean ± S.E. $^{**}P<0{\cdot}01$.

Virological examinations. Samples of pneumonic lesions were taken aseptically and placed in a virus transport medium. Monolayer cultures of rabbit kidney and equine fetal kidney cells were inoculated with homogenized tissues and examined for cytopathogenic effects over a 7-day period (Sugiura *et al.*, 1989). Respiratory viruses screened by this method included equine herpesvirus, rhinovirus and adenovirus.

Statistical evaluation. The results obtained in the experiments were analysed by Student's *t*-test.

Results

Experiment 1

Clinical findings. Thirteen of the 29 horses developed clinical signs of respiratory disease after 14 h of transport, but these had disappeared by the following day.

The numbers of circulating leucocytes and neutrophils in the horses with clinical signs of respiratory disease ("affected" horses) were significantly increased after transportation. There was also a tendency for the numbers of leucocytes and neutrophils to increase in the horses without clinical signs of respiratory disease ("non-affected" horses) after transportation (Fig. 2). In both affected and non-affected horses, the leucocyte and neutrophil counts one day after transport tended to return to the value before transport (Fig. 2).

Serological findings. During the 2-month period of monitoring, the titres to equine herpesvirus type 1, equine adenovirus, equine rhinovirus and calf diarrhoeal coronavirus rose in some cases, but the increases were less than four-fold.

Experiment 2

Environmental changes. The air temperature, relative humidity, airborne dust concentration and gaseous ammonia accumulation values recorded in the

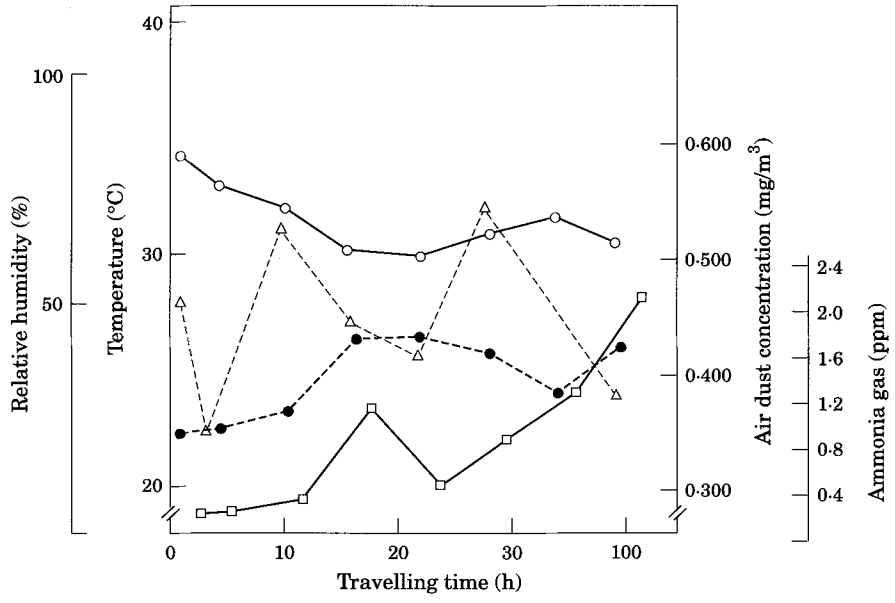


Fig. 3. Environmental changes in the vehicle during transportation. ●, Temperature; ○, relative humidity; △, atmospheric dust concentration; □, concentration of atmospheric ammonia.

truck while it was moving were 24–34°C, 58–85%, 0·29–0·60 mg/m^3 and 0·2–2·1 ppm respectively (Fig. 3). High ambient temperatures and low relative humidities were observed during the middle of the journey. The ammonia concentrations tended to increase as the journey proceeded.

Clinical findings. Twenty hours after the start of the journey three horses (nos 2, 3 and 6) out of the eight began to show clinical signs of respiratory disease, including pyrexia (Fig. 4), the peak rectal temperatures varying between 39·2°C and 39·9°C.

The results of endoscopical examination are shown in Table 1. In horses 3 and 6 ("affected") and 1 and 7 ("non-affected") tracheal mucopus increased after transport. Just before departure, the value of this indicator of tracheal inflammation was higher in horses that subsequently became affected than in those that did not.

The leucocyte and neutrophil counts in the three affected horses increased concurrently with the onset of pyrexia. The leucocyte count increased by 21–72% and the neutrophil count increased 2·0- to 2·7-fold (Fig. 5). On the other hand, in the five non-affected horses, the leucocyte and neutrophil counts increased only slightly with travel.

Pathological, histopathological, electron microscopical and immunohistochemical findings. In the three affected horses the cranio-ventral region of the right caudal lung lobe and accessory lobe had small, well-defined areas of dark consolidation with a homogeneously dark red colour on the visceral and cut

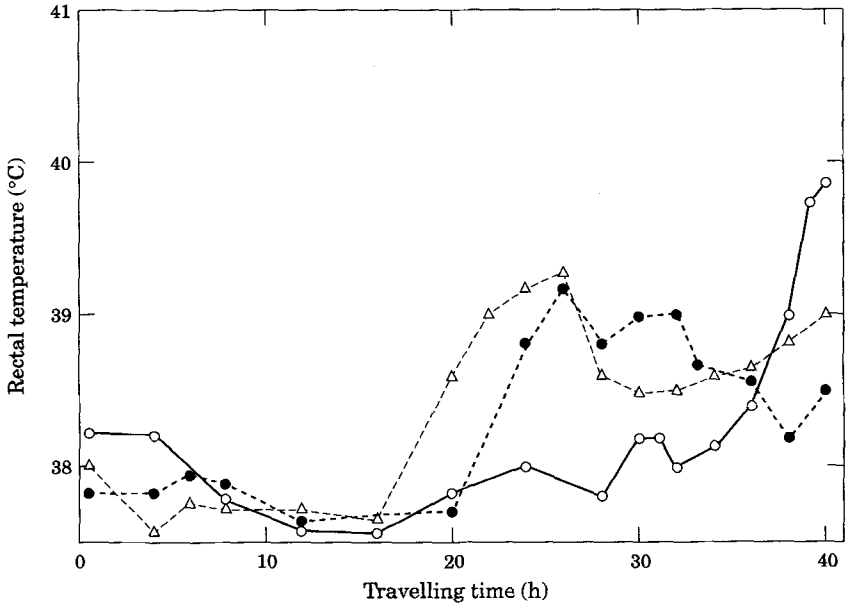


Fig. 4. Rectal temperatures of horses with pyrexia during transportation. △, Horse no. 2; ○, horse no. 3; ●, horse no. 6.

surfaces (Fig. 6; Table 2). The pleura and interlobular septa in these lesions were distended with oedema. No other macroscopical lesions were seen.

Histologically, the pulmonary lesions seen in the three affected horses consisted of serous neutrophilic bronchopneumonia and lobular pneumonia involving the respiratory bronchioles and surrounding alveoli (acentri–acinar distribution pattern) (Fig. 7). The terminal and respiratory bronchioles contained serous exudate, degenerating neutrophils, necrotic detritus and occasionally plant material, and their epithelia were swollen and pyknotic (serous neutrophilic bronchiolitis) (Fig. 7). The alveoli were filled with serous exudate, erythrocytes, fibrin, degenerating neutrophils and necrotic debris (serous neutrophilic intra-alveolar pneumonia). There was increased interalveolar septal thickness due to microvascular congestion, pneumocyte swelling and neutrophilic infiltration. The associated pleura and interlobular septa were dilated, with oedema, neutrophilic infiltration and haemorrhage.

Immunohistochemically, *S.z.* antigen was detected in the cytoplasm of neutrophils as a coarse granular deposit and in macrophages as diffusely dispersed fine particles, in the alveoli of the pneumonic lesions (Fig. 8).

Ultrastructurally, alveolar spaces contained neutrophils, oedema fluid evident as a fine granular precipitate, erythrocytes, strands of fibrin, material originating from lamellar inclusions, and cellular detritus (Figs 9 and 10). Neutrophils frequently exhibited severe signs of degeneration such as karyolysis and loss of cytoplasmic organization. Both types of pneumocyte in the pneumonic lesions showed oedematous swelling (Figs 9 and 10). Prominent pericapillary oedema producing widening of the interstitial spaces and separation

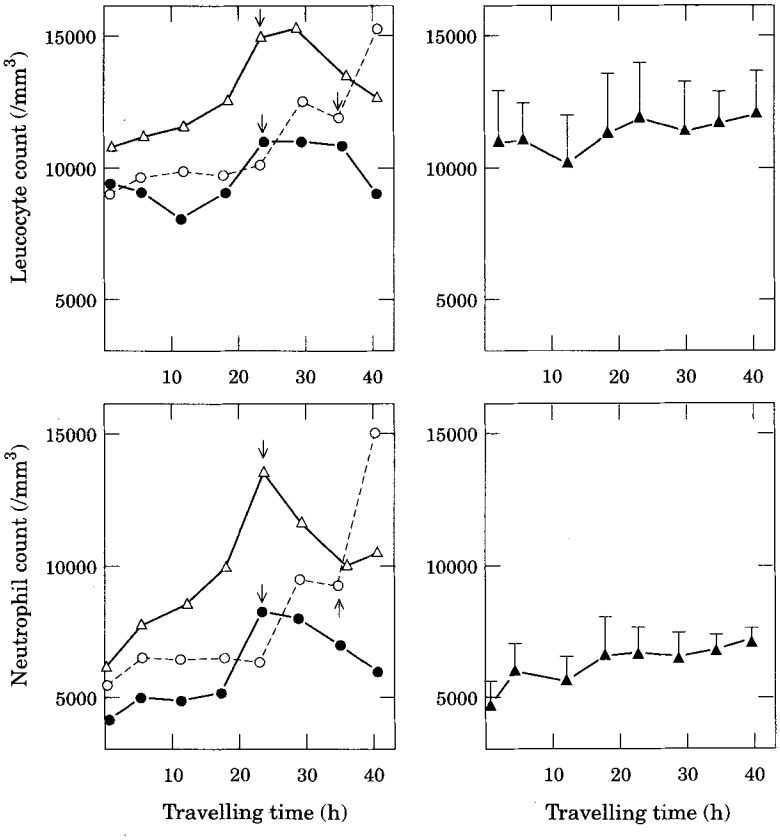
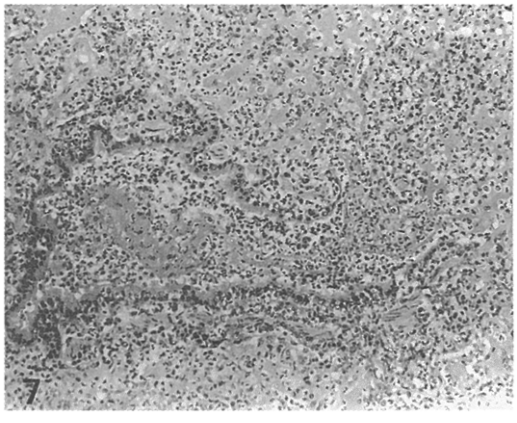


Fig. 5. Changes in white blood cell count associated with transportation. △, Horse no. 2; ○, horse no. 3; ●, horse no. 6; ↑, onset of pyrexia; ▲, horses without pyrexia ($n=5$). Values are expressed as the mean ± S.E.

of the connective tissue elements was seen (Figs 9, 10 and 11). Interalveolar septal capillaries were often packed with degranulated and swollen platelets, which were closely associated with endothelial surfaces, and neutrophils (Figs 10 and 11). The endothelial cells of the alveolar capillaries were swollen and vacuolated (Figs 9, 10 and 11). Occasionally, necrotic endothelial cells, and disorganized and disintegrated erythrocytes in capillaries were observed. No prominent changes were seen in non-pneumonic regions in the affected and non-affected horses.

The conspicuous microscopical changes in the tracheas of the affected horses were those of neutrophilic tracheitis (Table 2, Figs 12 and 13). The cilia showed a rough-surfaced and deformed pattern and clubbed and blunted ends (Fig. 12A). The lesions were accompanied by a slight to moderate granulomatous response in the lamina propria (Fig. 12B). The contents of the

Fig. 6. A small area of dark consolidation in the cranioventral region of right caudal lobe (arrow). Horse no. 2.



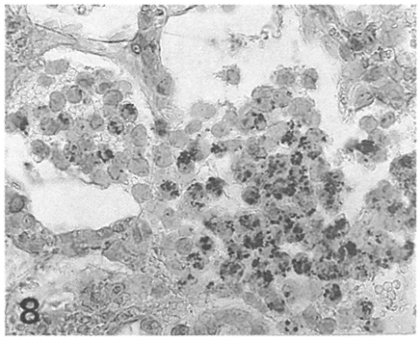


Fig. 7. Serous neutrophilic bronchopneumonia. Horse no. 3. HE. × 124. Horse no. 2.
Fig. 8. *Streptococcus equi* subsp. *zooepidemicus* antigen seen in the cytoplasm of neutrophils. Horse no. 2. ABCIT. × 292.

secretory granules in goblet cells and the glandular epithelium of tracheal glands were decreased (Fig. 12C).

In two of the affected horses (nos 2 and 3), mild lacunar palatine tonsillitis was observed in the region of thinning of the tonsillar epithelium (lymphoepithelial symbiosis region) (Table 3).

No histological lesions were seen in other major visceral organs.

Microbiological findings. *S.z.* was isolated in pure culture from the pulmonary lesions of the right lobe and accessory lobe of all three affected horses (Table 2). Specimens taken from normal lung tissue in the affected and non-affected horses failed to yield bacterial growth. *S.z.* was isolated as the predominant organism from the entire length of the trachea in two of the affected horses (nos 2 and 3), with a tendency for the number of bacteria isolated to decrease

Table 2
Distribution of pathological lesions and bacterial isolates according to sampling site

Site	*Pathological lesion* / bacterial isolates† in horse no.*							
	1	*2‡*	*3‡*	*4*	*5*	*6‡*	*7*	*8*
Upper trachea	−/0	+++/2×10^2	+++/3×10^5	−/0	+/70	++/1×10^2	−/0	−/0
Middle trachea	−/0	++/1×10^2	+++/5×10^4	−/0	+/0	+/0	−/0	−/0
Lower trachea	−/0	+/0	+/1×10^2	−/0	−/0	−/0	−/0	−/0
Right lung								
Principal bronchus	−/0	−/0	−/10	−/0	−/0	−/50	−/0	−/0
Cranial lobe	−/0	++/1×10^2	−/0	−/0	−/0	−/0	−/0	−/0
Craniodorsal portion of caudal lobe	−/0	−/0	−/0	−/0	−/0	−/0	−/0	−/0
Cranioventral portion of caudal lobe	−/0	+++/1×10^3	+++/2×10^2	−/0	−/0	+++/7×10^2	−/0	−/0
Caudodorsal portion of caudal lobe	−/0	−/0	−/0	−/0	−/0	−/0	−/0	−/0
Caudoventral portion of caudal lobe	−/0	−/0	++/1×10^2	−/0	−/0	−/0	−/0	−/0
Accessory lobe	−/0	++/1×10^2	−/0	−/0	−/0	−/0	−/0	−/0
Left lung								
Principal bronchus	−/0	−/0	−/30	−/0	−/0	−/0	−/0	−/0
Other sites	−/0	−/0	−/0	−/0	−/0	−/0	−/0	−/0

* −, Negative; +, slight; ++, moderate; +++, severe.
† Predominant isolates were *Streptococcus equi* subsp. *zooepidemicus*. Values are expressed as viable colony counts in tissues or fluids (per g or ml).
‡ Horses with pyrexia.

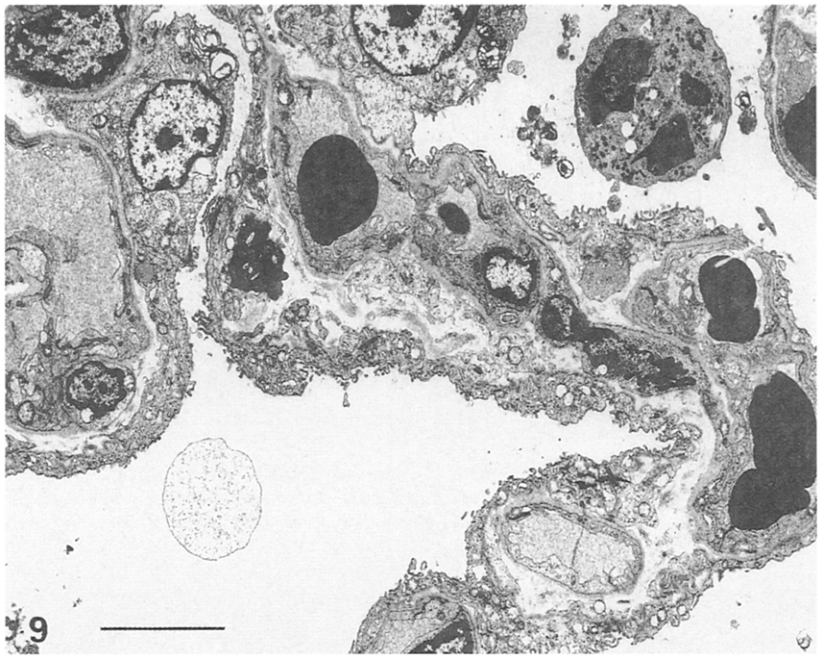
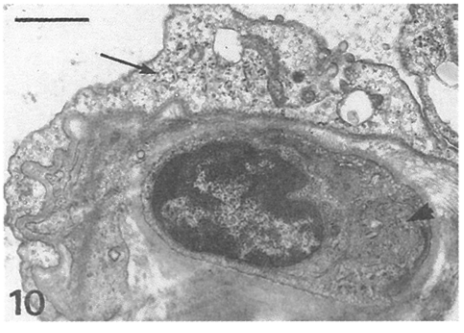


Fig. 9. Alveolar spaces containing neutrophils, cellular debris and material originating from lamellar inclusions. Pericapillary oedema and oedematous swelling of pneumocytes are prominent. Horse no. 3. EM. Bar = 5 μm.



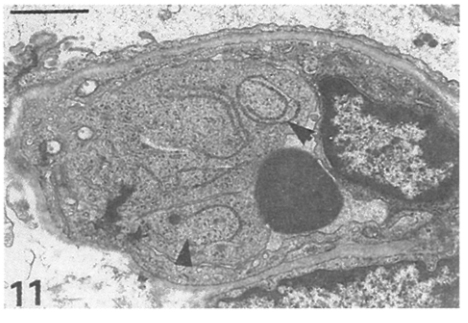


Fig. 10. A type 1 pneumocyte showing oedematous swelling (arrow). A platelet occludes an alveolar capillary (arrowhead). Pericapillary oedema is seen. Alveolar space contained oedema fluid evident as fine granular precipitate. Horse no. 2. EM. Bar = 1·4 μm.

Fig. 11. Platelets occluding an alveolar capillary (arrowheads) and pericapillary oedema. Platelets were degranulated, swollen and closely juxtaposed to endothelial cells. Horse no. 3. EM. Bar = 1 μm.

from the upper to the lower trachea (Table 2). In one (no. 5) of the five non-affected horses, a slight growth of *S.z.* was obtained from the upper trachea. No bacteria were recovered from the other visceral organs or from the pleural and peritoneal fluids.

Mixed bacterial growth was obtained from the palatine tonsils of affected and non-affected horses (Table 3), but the number of *S.z.* isolated was higher in the former than in the latter. The ratio of the bacterial count of *S.z.* to

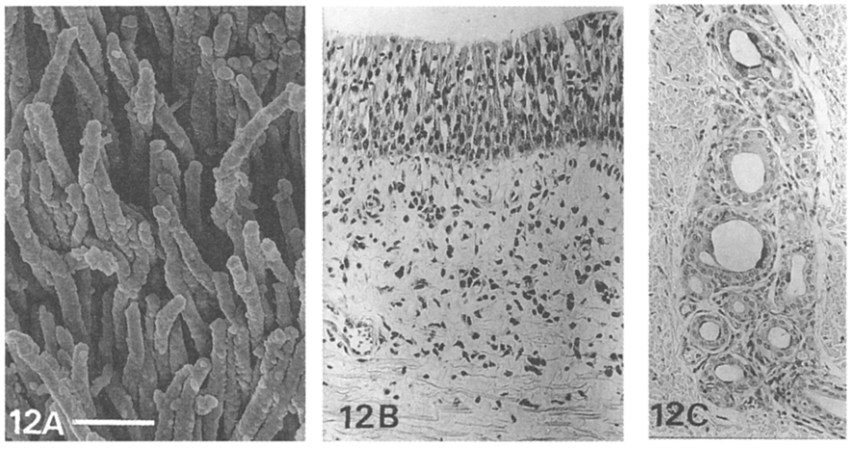


Fig. 12. Trachea of an affected horse (no. 3). (A) Cilia with clubbed and deformed ends. SEM. Bar = 1·0 μm. (B) Neutrophilic tracheitis accompanied by granulomatous inflammation in the lamina propria. HE. × 175. (C) Tracheal glandular epithelium with decreased secretory granules (cf. Fig. 13C). PAS. × 152.

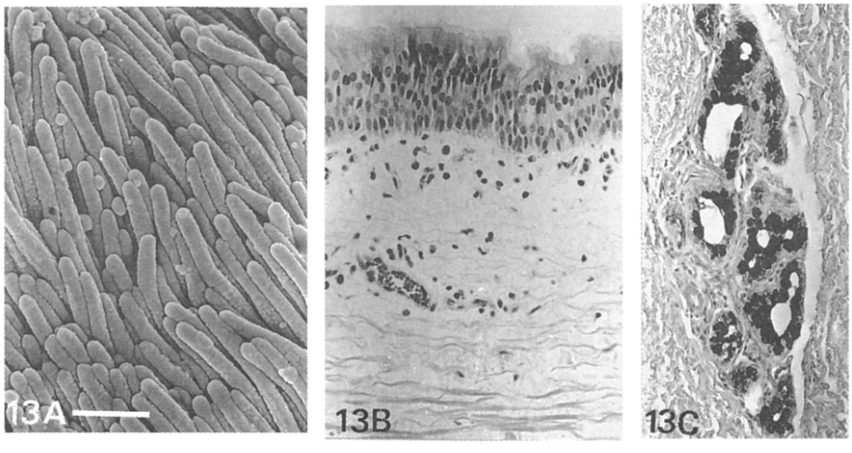


Fig. 13. Trachea of a non-affected horse (no. 5). (A) Closely packed cilia with relatively smooth surface. SEM. Bar = 1·0 μm. (B) Lamina propria with slight cellular infiltration and oedema. HE. × 175. (C) Tracheal glandular epithelium rich in secretory granules. PAS. × 152.

that of other bacterial species in the palatine tonsils of affected horses ranged from 1:2 to 1:5. This ratio in the non-affected horses ranged from 1:20 to 1: 300.

Tissues from pneumonic lesions proved negative for respiratory viruses.

Discussion

It is likely that transport adversely affected the normally effective mucosal defence mechanism in the airways, leading to invasion by *S.z.* (a common commensal micro-organism in the equine tonsil and nasopharynx) into the lower airways, thus inducing acute lower airway inflammation in the affected

Table 3
Histological lesions and predominant bacteria in palatine tonsils

Bacterial species	*Viable counts (per g) in tonsil of horse no.*							
	1 (+)	*2** (++)	*3** (+)	*4* (+)	*5* (+)	*6** (+)	*7* (+)	*8* (+)
S.z.	2×10^3	1×10^6	3×10^5	1×10^4	2×10^2	1×10^5	2×10^3	1×10^4
α-Haemolytic streptococcus	1×10^4	1×10^6	—	1×10^5	1×10^4	2×10^5	1×10^5	2×10^5
Escherichia coli	5×10^3	2×10^6	—	—	—	—	—	3×10^3
Others†	1×10^5	1×10^6	3×10^5	1×10^5	5×10^4	4×10^4	1×10^5	1×10^4
All (total)	11×10^4	5×10^6	6×10^5	2×10^5	6×10^4	3×10^5	2×10^5	2×10^5
Ratio of *S.z.* count to total viable colony count (%)	1·8	20	50	5	0·3	33	1	5

(+), Slight tonsillitis; (++), moderate tonsillitis.
S.z., *Streptococcus equi* subsp. *zooepidemicus*.
* Horses with pyrexia.
† Mainly *Staphylococcus* spp., *Enterococcus* spp. and *Bacillus* spp.

horses. Evidence supporting this possibility included (a) the abrupt onset of neutrophilic leucocytosis with an increase of both the absolute and relative number of neutrophils, associated with transport, (b) increased mucopus in the trachea after transport, (c) spread of *S.z.* from the upper to the lower trachea, where the organism would not normally be expected (Blunden and Mackintosh, 1991), associated with neutrophilic tracheitis, (d) tracheal ciliary abnormality suggestive of impairment of mucociliary function in the airway, (e) a decrease in the amount of secretory granules in goblet cells and the glandular epithelium of the trachea (decreased mucous lining), and (f) acute pneumonic lesions containing *S.z.* and *S.z.* antigen. Endoscopical and pathological findings in the trachea indicated that airway inflammation was initially present in some of the horses and was exacerbated by transport. These findings suggest that pre-existing mild airway infection leads to pneumonia when horses are subjected to a prolonged period of transportation, as has been claimed by Raphael and Beech (1982). There seemed to be an association between the spread of *S.z.* in the airways and the presence of the organism in the palatine tonsil, with lacunar tonsillitis, in the affected horses. It has been reported that increased exposure of the lower respiratory tract to pathogenic micro-organisms under conditions of environmental stress is accompanied by increased colonization of the palatine tonsil by agents such as *Pasteurella* spp. in lambs and sheep (Yates, 1988). It is difficult to explain the association between transport and diminished mucosal defence mechanisms in the airways. One possible explanation is that the desiccating effects of exposure to air currents and low relative humidity while the transport vehicle is in motion (Leadon, 1994) may reduce the thickness of the respiratory mucous lining and prevent effective ciliary motility (Derksen, 1991). The finding that the tracheal membrane surface was not glistening and moist and that the cilia often showed a

deformed pattern with clubbed ends suggested that drying of mucous membranes had occurred. A further possibility is that a large number of airborne hay dust particles inhaled during transport may have suppressed the mucosal clearance capability of the airways, as plant material was detected frequently in the inflammatory exudates in the airways. A similar association was reported by Chrisman (1987).

Possible infection with unidentified viruses, mycoplasmata (Gerber, 1986) and chlamydiae (Burrell *et al.*, 1986) as primary pathogens of equine respiratory infection cannot be ruled out, but in the present study the findings indicated that *S.z.* was the primary cause of lower airway inflammation. Profuse migration of neutrophils in the lung seemed to develop as a result of *S.z.* infection, suggesting that injury to pneumocytes and endothelial cells may be caused in part by tissue-damaging enzymes released from these degenerating cells (Yates, 1988). The leucocyte response in the peripheral blood seems consistent with this finding. Platelets closely associated with the endothelial surface might enhance neutrophil adhesion and congestion of the microvasculature, leading to vascular damage, increased capillary permeability (Jorgensen *et al.*, 1970), intra-alveolar effusion, and pleural and interlobular septal oedema.

Pathological and microbiological examination revealed a predominantly cranioventral distribution of pneumonic lesions suggestive of increased bacterial deposition in the lesions, possibly leading to impaired clearance and bacterial settling due to gravitational influence (Dungworth, 1991). The propensity of the horse to develop right-sided lesions, which have been reported in inhalation pneumonia and lung abscesses associated with *S.z.* infection (Rooney, 1991), may be attributable to the ramification of the right principal bronchus, which passes caudolaterally with a relatively straight continuation of the trachea as compared with the left principal bronchus, which runs at a more acute angle. The latter has an inside diameter slightly larger than that of the right principal bronchus (Wada *et al.*, 1992).

Similarities were noted between the pathological nature of the pneumonia in the present study and that of the transport-associated pneumonia described by Oikawa *et al.* (1994). However, compared with the former, the extent and severity of the latter pneumonia were severe, clumps of *S.z.* were frequently seen in the pneumonic lesions, and the viable counts of *S.z.* in the lesions were strikingly higher. Further research is required to ascertain whether such differences reflect differences in exposure of the pulmonary parenchyma to *S.z.*, the virulence of *S.z.*, or susceptibility of the lung to profuse bacterial growth.

References

Bayly, W. M., Liggit, H. D., Huston, W. and Laegreid, W. W. (1986). Stress and its effect on equine pulmonary mucosal defenses. *Proceedings of the 32nd Annual Convention of the American Association of Equine Practitioners*, 253–262.

Blunden, A. S. and Mackintosh, M. E. (1991). The microflora of the lower respiratory tract of the horse: an autopsy study. *British Veterinary Journal*, **147**, 238–250.

Burrell, M. H. (1985). Endoscopic and virological observations on respiratory disease in a group of young Thoroughbred horses in training. *Equine Veterinary Journal*, **17**, 99–103.

Burrell, M. H., Chalmes, W. S. K. and Kewley, D. R. (1986). Isolation of *Chlamydia psittaci* from the respiratory tract and conjunctiva of thoroughbred horses. *Veterinary Record*, **119,** 302.

Chrisman, M. V. (1987). Effects of transport on pulmonary mucosal defenses of horses. Master's thesis, Washington State University, Pullman, USA.

Derksen, F. J. (1991). Applied respiratory physiology. In: *Equine Respiratory Disorders*, J. Beech, Ed., Lea and Febiger, Philadelphia, pp. 18–20.

Dungworth, D. L. (1991). Bronchopneumonia. In: *Pathology of Domestic Animals*, 4th Edit., Vol. 2, K. V. F. Jubb, P. C. Kennedy and N. Palmer, Eds, Academic Press, San Diego, pp. 591–592.

Gerber, H. (1986). Contagious equine pleuropneumonia. In: *Equine Diseases*, H. J. Wintzer, Ed., Verlag Paul Parey, Berlin and Hamburg, pp. 38–39.

Hayakawa, Y., Komae, H., Ide, H., Nakagawa, H., Yoshida, Y., Kamada, M., Kataoka, Y. and Nakazawa, M. (1993). An occurrence of equine transport pneumonia caused by mixed infection with *Pasteurella caballi*, *Streptococcus suis* and *Streptococcus zooepidemicus*. *Journal of Veterinary Medical Science*, **55,** 455–456.

Jorgensen, L., Hovig, T., Roowsell, H. and Mustard, J. (1970). ADP-induced platelet aggregation and vascular injury in swine and rabbits. *American Journal of Pathology*, **61,** 161–176.

Leadon, D. P. (1994). Transport stress. In: *The Athletic Horse*, D. R. Hodgson and R. J. Rose, Eds, W. B. Saunders Co., Philadelphia, pp. 371–378.

Leadon, D. P., Daykin, J., Backhouse, W., Frank, C. and Atock, M. A. (1990). Environmental, hematological and blood biochemical changes in equine transit stress. *Proceedings of the 36th Annual Convention of the American Association of Equine Practitioners*, 485–490.

Mair, T. S. and Lane, J. G. (1989). Pneumonia, lung abscesses and pleuritis in adult horses: a review of 51 cases. *Equine Veterinary Journal*, **21,** 175–180.

Oikawa, M., Kamada, M., Yoshikawa, Y. and Yoshikawa, T. (1994). Pathology of equine pneumonia associated with transport, and isolation of *Streptococcus equi* subsp. *zooepidemicus*. *Journal of Comparative Pathology*, **111,** 205–212.

Raphael, C. F. and Beech, J. (1982). Pleuritis secondary to pneumonia or lung abscessation in 90 horses. *Journal of the American Veterinary Medical Association*, **181,** 808–810.

Rooney, J. R. (1991). Pneumonia. In: *Equine Respiratory Disorders*, J. Beech, Ed., Lea and Febiger, Philadelphia, p. 149.

Sugiura, T., Matsumura, T. and Fukunaga, Y. (1989). Isolation and identification of viruses from racehorses with pyrexia. *Bulletin of Equine Research Institute*, No. 26, 53–59.

Traub-Dargatz, J. L., McKinnon, A. O., Bruyninckx, W. J., Thrall, M. A., Jones, R. L. and Blancquaert, A-M. B. (1988). Effect of transportation stress on bronchoalveolar lavage fluid analysis in female horses. *American Journal of Veterinary Research*, **49,** 1026–1029.

Wada, R., Aida, H., Kaneko, M., Oikawa, M., Yoshihara, T., Tomioka, Y. and Nitta, M. (1992). Identification of the bronchi for bronchoscopy in the horse and segmentation of the horse lung. *Japanese Journal of Equine Science*, **3,** 37–43.

Yates, W. D. G. (1988). Pneumonia. In: *Special Veterinary Pathology*, R. G. Thomson, Ed., B. C. Decker Inc., Toronto, pp. 97–98, 103–104.

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