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SHORT COMMUNICATION

THE EFFECTS OF 23.5 PPM FORMALDEHYDE VAPOUR ON TRACHEAL EPITHELIUM OF HATCHING CHICKS

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SUMMARY

The effects of 23.5 parts per million (ppm) formaldehyde vapour on the epithelial lining of embryonated eggs and hatching chicks were examined by the scanning electron microscopy. The 19 and 20-day-old embryonated eggs as well as day-old chicks were divided into fumigated and non-fumigated groups. The fumigated chicks were exposed to 23.5 ppm formaldehyde vapour for 6, 30 and 54 h whereas the non-fumigated groups were used as control. Tracheal samples were collected one day after the formaldehyde treatment. Scanning electron microscopy of treated groups revealed surface morphological changes including accumulation of mucus, deciliation and epithelial sloughing. Lesions were more severe in chicks exposed to formaldehyde vapour for 54 h compared to chicks exposed to formaldehyde vapour for 6 h and 30 h. On the other hand, no lesions were observed in the trachea of chicks from the untreated group.

Keywords: Tracheal epithelium, formaldehyde vapour, hatching chicks

INTRODUCTION

Formaldehyde, a powerful antiseptic, germicide and fungicide is a colourless gas with a strong pungent odour (Wills, 1993). It is soluble in ethanol and water. It was widely used as a preservative for biological specimens before being replaced by other preservatives because of health risks. Nevertheless, formaldehyde is still commonly use as a fumigant in most commercial hatcheries since it helps to reduce microbial contamination in hatching eggs and hatcheries (Beesley, 1980; Deeming, 1992).

Continuous exposures to formaldehyde vapour create problems to the hatchery staffs and chicks. In chicks, the respiratory tract is damaged as formaldehyde vapour of low (10.9 ppm) and high (130 ppm) concentrations destroy the epithelial lining (Sander *et al.*, 1995; Fauziah *et al.*, 1996). In Malaysian commercial hatcheries, formaldehyde at the concentration of 23.5 ppm is used as a fumigant. Therefore, this study was undertaken to investigate the effects of 23.5 ppm formaldehyde vapour on the tracheal epithelium of hatching chicks in one of the commercial hatcheries in Malaysia.

MATERIALS AND METHODS

Formaldehyde exposure

A total of 23,040 eggs were used in this study. On day 18 of incubation, 11,520 eggs were transferred from a setter into a 11.0 m^3 hatcher (Jamesway®) at 37°C and 70% relative humidity. These eggs were then divided into 3 groups. Groups A, B and C were exposed to formaldehyde vapour for 6, 30 and 54 h respectively. The formaldehyde vapour was liberated from 2 metal containers containing 300 ml of 40% formalin at day 19 of incubation. After half and hour of fumigation, the concentration of formaldehyde vapour was measured as 23.5 ppm using a formaldehyde meter (Dräger Pac III). Equal numbers of eggs in other hatcher were not exposed to formaldehyde vapour and treated as control.

Tracheal collection and processing

Five eggs from each group (A, B, C and control) were randomly collected on days 19 and 20 of incubation. Five day-old chicks from each group were also randomly collected. The un-hatched eggs and day-old chick were killed with an overdose of sodium pentobarbitone, administered intraperitonially before tracheal samples (1 cm^3) were immediately collected for scanning electron microscopy (SEM).

Preparation of tracheal samples for SEM

Tracheal samples were initially fixed in 4% buffered gluteraldehyde for 24 h and then in 1% buffered osmium tetraoxide for 2 h at 4°C. Later, the samples were processed routinely for SEM.

All specimens were examined under a Jeol 6400 SEM, at an accelerating voltage of 15kV. The lesion scoring was carried out according to the criteria described by Fauziah *et al.* (1996).

Statistical analysis

Data were subjected to analysis of variance with the aid of General Linear Models (GLM) of SAS® software (SAS® Institute, 1991). Statistical significance was considered at $P \leq 0.05$.

RESULTS

Lesion score

Lesion scores for the tracheal epithelium of control and treated chicks are shown in Table 2. There was a significant difference ($p \leq 0.05$) in lesion scores between fumigated and non-fumigated groups. Within the fumigated group, the duration had significant effect on lesion score. Day-old chicks showed the highest lesion score of 2.52 compared with 20-day-old and 19-day-old embryonated eggs with average lesion scores of 1.78 and 1.30 respectively. In 19-day-old embryo and 1-day-old chicks of the control group, average lesion scores were 0.20 and 0.40 respectively.

Table 1: Mean lesion scores for the tracheal epithelium of chicks exposed to formaldehyde vapour (mean ±SEM)

Exposure time (h)	Control	Treated
6	0.20±0.01*	1.30±0.56[a]
30	0.00±0.00*	1.78±0.10[b]
54	0.40±0.04*	2.52±0.14[c]

[a,b,c] Means within a column with different superscript differ significantly ($p \leq 0.05$)

*Significant differences ($p \leq 0.05$) between means within a row

Scanning electron microscopy

SEM revealed injuries to the airway epithelium of the embryos and newly hatched chicks exposed to formaldehyde vapour. The luminal surface of trachea exposed to 23.5 ppm of formaldehyde exhibited areas of excessive accumulation of mucus, matted cilia, de-ciliation (Fig. 1) and focal desquamation of epithelium (Fig. 2). Tracheal tissues of the non-exposed control chicks appeared normal (Fig. 3).

DISCUSSION

In the present study, an exposure to 23.5 ppm formaldehyde vapour for 54 h resulted in more severe damage to the respiratory epithelium compared to 6 and 30 h of formaldehyde exposure. The epithelial linings of the trachea of chicks exposed to formaldehyde vapour exhibited areas of excessive accumulation of mucus, matted cilia, de-ciliation and focal desquamation of the epithelium. These findings were similar to those of hatching chicks exposed to low level of formaldehyde (Fauziah *et al.*, 1996) as well as high levels of formaldehyde (Furuta *et al.*, 1989; Sander *et al.*, 1995). Exposure to 10.9 ppm formaldehyde vapour observed by Fauziah *et al.* (1996) revealed clumping of cilia, blebs on the cilial wall, de-ciliation and also exfoliation of the epithelium. Sander *et al.* (1995) reported that exposure of tracheal epithelial lining to 130 ppm formaldehyde vapour resulted in blunt cilia and blebs on the surface of cilia. These authors suggested that the different degrees of adverse effect generated by formaldehyde vapour in the airways corresponds to the concentration and duration of exposure to formaldehyde vapour.

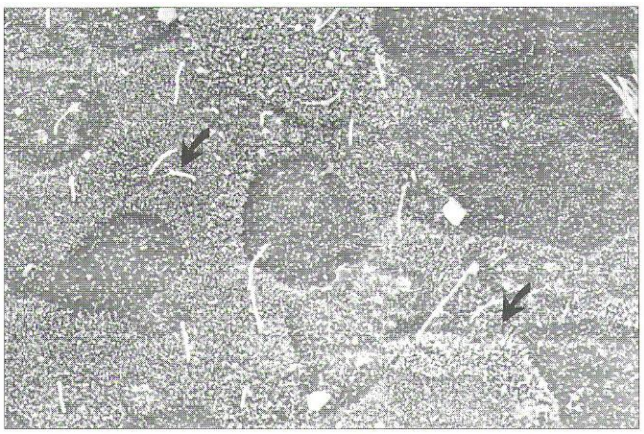

Fig. 1. Scanning electron micrograph of the luminal surface of the trachea of a day-old chicken exposed to formaldehyde vapour. Note large area of de-ciliation (arrows). x3,000

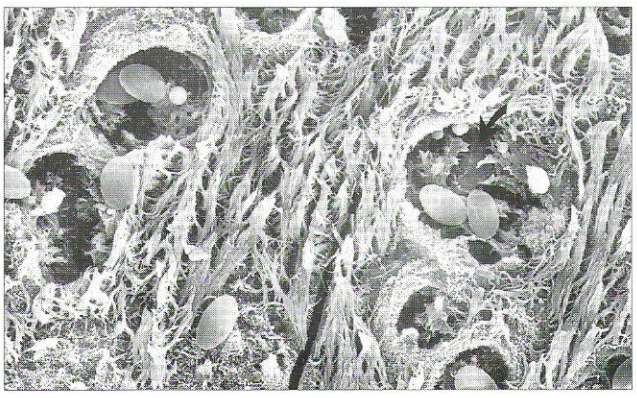

Fig. 2. Scanning electron micrograph of the respiratory epithelial surface of a day-old chick exposed to formaldehyde vapour. Note the multifocal desquamation of the epithelium (arrow). x1,200

The adverse effect of formaldehyde vapour resulted in large areas of de-ciliation and excessive mucus secretion from the goblet cells. These alterations lead to matting of cilia and thus, reduce the motility of the cilia (Sander *et al.*, 1995). Other noxious gases such as ammonia can also cause irritation to the respiratory system of chicks (Al-Mashhadani and Beck, 1985). Inevitably, these cilia are unable to clear the mucus and therefore, cause an obstruction to the airflow. The affected chicks will suffer from dyspnoea and microbial infection due to accumulation of mucus, which acts as a growth medium for microbes to flourish.

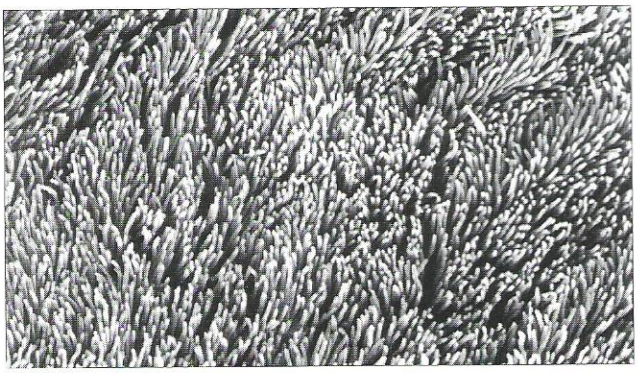

Fig. 3. Scanning electron micrograph of the trachea from a 20-day-old chick not exposed (control) to the formaldehyde vapour. x4300

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RINGKASAN

KESAN 23.5 PPM WAP FORMALDEHID KE ATAS EPITELIUM TRAKEA ANAK AYAM BARU MENETAS

Kesan 23.5 ppm wap formaldehid ke atas lapisan epitelium telur bembrio dan anak ayam baru menetas dikaji secara mikroskopi elektron imbasan. Telur terembrio berumur 19 dan 20 hari berserta anak ayam berumur satu hari dibahagikan kepada kumpulan terfumigat dan bukan terfumigat. Anak ayam terfumigat didedahkan kepada wap 23.5 ppm formaldehid selama 6, 30, dan 54 jam manakala yang bukan terfumigat bertindak sebagai kawalan. Sampel trakea diambil sehari selepas rawatan formaldehid. Mikroskopi elektron imbasan kumpulan rawatan menunjukkan perubahan permukaan merangkumi pengumpulan mucus, penyasiliaan dan pelpuhan epitelium. Lesi yang lebih teruk dilihat pada anak ayam yang didedahkan kepada wap formaldehid selama 54 jam.