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AIRBORNE ENDOTOXINS AND AIRBORNE GRAM-NEGATIVE BACTERIA IN CHINESE RABBIT HOUSES

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Introduction

Global rabbit production is currently estimated at more than 1 million tones per year, according to the FAO. The world's largest producer is China with 315.000 tones in 2000 (FAO, 2001).

One major problem in rabbit meat production is infectious diseases of the respiratory tract. Usually respiratory infections are due to an association of non-specific contributing causes with infectious agents. Important non-specific contributing factors are the concentration of toxic gases, especially ammonia, and the dust concentration. Further the concentration of airborne endotoxins seems to play an important role in the development of respiratory diseases. Chronic exposure to airborne endotoxins can compromise several defense mechanisms of the host (Deeb and DiGiacomo, 2000; Halloy et al., 2005).
In this study airborne endotoxin concentrations, the number of total airborne aerobic bacteria and the number of airborne gram-negative bacteria were measured in 3 rabbit houses. Further the species composition of the isolated airborne gram-negative bacteria was studied.

Material and methods

- Rabbit houses studied

Three rabbit houses, two housing breeding rabbits and one housing broiler rabbits were studied. A description of these animal houses is given in Table 1.

- Determination of airborne endotoxins and airborne bacteria

The sampling devices were located near the middle of the animal houses 1.5 m above the ground. All air samples were collected during normal work periods in all animal houses. Animal disturbance during sampling was strictly avoided. Outside air samples were collected windward at a distance of 5 m from the animal houses.
- Airborne endotoxins
Airborne endotoxin concentrations were determined using AGI-30 impinger and the Limulus Amebocyte Lysate (LAL) assay as described by Zucker et al. (2000).

- Airborne bacteria

Six-stage Andersen samplers (Andersen, 1958) were used to determine the concentration of airborne aerobic and airborne aerobic gram-negative bacteria. The samplers were equipped with 5% sheep blood agar plates and operated for 1 to 5 min with an air flow rate of 28.3 l/min. The exposed sheep blood agar plates were incubated at 37°C for 24 h. Than the number of grown colonies was counted and the positive whole correction was applied.

- Assessment of gram-negative bacterial flora

From each animal house on each sampling day 2 sets of agar plates from an Andersen sampler (2 x 6 plates) were used to identify gram-negative bacterial colonies. All grown colonies were screened for their Gram reaction using the "KOH assay" (Burkhardt, 1992). Gram-negative colonies were subcultured and their species were identified by using the api 20 E and the api 20 NE system (Bio Merieux, Marcy-I'Etoile, France).

Table 1: Description of studied animal houses

Rabbit house	Layout	Size	Animal places	Feed	Ventilation	T in °C	RF in %
A	Two-tier breeding cage unit	55m x 32m x 5m	240 mature 1000 infants	pellets	Fan forced ventilation	**10-20**	**60-80**
B	Three-tier breeding cage unit	24m x 6.5m x 32m	200 mature 1000 infants	pellets	Window ventilation	15-23	48-57
C	Three-tier broiler cage unit	18m x 5m x 3.7m	1200 broiler rabbits	pellets	Window ventilation	16-25	65-80

T = temperature, RF = relative humidity

Results and discussion

The present experiments were designed to obtain data on concentrations of airborne bacteria and endotoxins in rabbit houses. Furthermore the species composition of the airborne gram-negative bacterial flora was characterized.

Concentrations of inhalable endotoxins ranged from 22 to 774 EU/m³ (Table 2). These are similar concentrations of airborne endotoxin usually found in cattle stables, but considerable smaller concentrations than those found in poultry or pig houses (Takai and Pedersen, 2002). The measured airborne endotoxin levels did not exceed a suggested no effect level for the toxic pneumonitis in men (2000 EU/m³) but exceeded a suggested no effect level for airways inflammation (100 EU/m³) in part (Rylander, 1997). However, it should be considered that the concentrations of airborne endotoxins were estimated only during normal work periods in all rabbit houses. Airborne endotoxin concentrations could be expected to be grater during special activities, like removal of faeces or stalling new animals.

The influence of the measured airborne endotoxin concentrations on the health of the housed rabbits is not known. There are no established no effect levels for airborne endotoxin concentrations concerning the respiratory health of rabbits. However, from experiments with pigs it is known that airborne endotoxins can promote the development of multifactorial respiratory diseases (Halloy et al., 2005).

In most outside air samples the endotoxin concentration was below the detection limit of our test system. The highest concentration in the outside air was 38.4 EU/m³.

The number of total airborne aerobic bacteria in the rabbit houses varied between 780 to 20100 CFU/m³, the number of airborne aerobic gram-negative bacteria between 39 to 1030 CFU/m³ (Table 2). That indicates, that only a small portion of culturable airborne aerobic bacteria in the rabbit houses were gram-negative. That is a similar relation as known from the airborne aerobic bacterial flora in other animal houses (Zucker et al., 2000a). In the outside air the concentration of airborne aerobic bacteria ranged from 490 to 8300 CFU/m³.

Table 2: Concentration of airborne Endotoxin and airborne bacteria in rabbit house A - C

Rabbit house	n	Airborne Endotoxin (x 10^2 EU/m³)			Airborne gram-negative aerobic bacteria (x 10^2 CFU/m³)			Airborne aerobic bacteria x 10^3 CFU/m³		
		Median	Max.	Min.	Median	Max.	Min.	Median	Max.	Min.
A	21	1.37	4.60	0.45	1.25	1.90	0.39	4.80	8.10	1.73
B	18	1.35	3.20	0.22	2.74	8.20	0.97	3.26	8.30	0.78
C	18	3.93	7.74	0.64	4.38	10.3	1.03	5.72	20.1	0.98

In all rabbit houses the *Enterobacteriaceae* clearly dominated the airborne gram-negative bacterial flora (Table 3). Within the family of *Enterobacteriaceae* the species *E. coli* was predominant in all rabbit houses (rabbit house A: 90.5%; rabbit house B: 92.5%, rabbit house C: 87.6%). Similar results are known from pig and poultry houses (Zucker et al., 2000a).

Within the *Neisseriaceae* the species *Neisseria mucosa* was found at a high frequency in all 3 rabbit houses (rabbit house A: 80.0%, rabbit house B: 57.1%; rabbit house C: 55.6%). Several species in the genus Neisseria, like *Neisseria mucosa*, occur as commensals on the mucous membranes of warm-blooded animals and humans, particularly on the nasopharynx and conjunctiva (Cartrer and Cole, 1990).

A broad spectrum of different species was found within the *Pseudomonadaceae*, including *Pseudomonas aeruginosa.* This organism has been reported responsible for wound infections and listed as an important nosocomial agent in humans and animals (Carter and Cole, 1990).

In two rabbit houses also a considerable amount of *Pasteurellaceae* (Table 3), with *Pasteurella multocida* as primary species, was found. In house A 76.5% of the isolated Pasteurellaceae were identified as *Pasteurella multocida*, in house C 84.5%, respectively. *Pasteurella multocida* is the most common respiratory pathogen of rabbits. Usually Pasteurellosis is associated with upper respiratory diseases, characterized by rhinitis with mucopurulent nasal discharge (Deeb and DiGiacomo, 2000). Mucopurulent nasal discharge was observed on several rabbits in animal house A and B during this investigation. However, in house C where the rabbits did not show clinical sings of respiratory diseases airborne *Pasteurella* ssp. was not detected.

Table 3: Composition of the airborne gram-negative bacterial flora in rabbit house A - C

Bacterial family	Rabbit house A	Rabbit house B	Rabbit house C
Enterobacteriaceae	64,3 %	84,2%	72,3%
Pseudomonadaceae	14,0%	9,7%	11,2%
Neisseriaceae	5,4%	5,5%	7,9%
Pasteurellaceae	18,3%	0,0%	8,6%
Others*	0,0%	0,6%	0,0%

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