Acta vet. scand. 1999, *40,* 197-203.

Effect of Aerial Ammonia on Porcine Infection of the Respiratory Tract with Toxigenic *Pasteurella multocida*

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Andreasen M, Bækbo P, Nielsen JP: Effect of aerial ammonia on porcine infection of the respiratory tract with toxigenic Pasteurella multocida. Acta vet scand. 1999, 40, 197-203. – The objective of the experimental study was to examine whether aerial ammonia alone could predispose the respiratory system of pigs to infection with toxigenic *Pasteurella multocida* type A. Two groups of 5 pigs each were continuously exposed to 50 ppm ammonia and less than 5 ppm ammonia, respectively, for a 59-day period (from 37 kg to 90 kg bodyweight) followed by necropsy. In an aerosol chamber all pigs were exposed to an aerosol of toxigenic *P. multocida* type A (mean bacterial concentration in the aerosol-exposure chamber: 10^5 colony forming units/m^3; exposure period: 25 min) at day 10, 21, 35 and 49 after the onset of ammonia exposure.

During the experiment none of the pigs showed clinical signs of pneumonia nor did they develop visible distortion of the snout. None of the pigs had gross lesions in the lungs at necropsy and toxigenic *P. multocida* was not detected by culture from the lungs from any of the pigs. The chance of recovering toxigenic *P. multocida* from nasal swabs (collected during experiment) was 2- 4 times greater in the test group compared to the control group. The average daily weight gain was lower for the ammonia exposed pigs compared to the control group.

In conclusion the results from this study suggest that ammonia in concentrations of 50 ppm is unlikely to predispose growing pigs to pulmonary infection with toxigenic *P. multocida.*

pigs; respiratory disease; aerosol exposure; nasal colonisation; weight gain.

Introduction

Pasteurella multocida is a common secondary pathogen in swine pneumonia and is normally considered incapable of colonising the lungs unless some predisposing damage has occurred (*Ciprián et al.* 1994). Also, colonisation of the nasal cavity is poor unless there is a pre-existing mucosal damage, which may be of chemical (eg. acetic acid) or infectious nature (e.g. *Bordetella bronchiseptica*) (*De Jong* 1992, *Chanter & Rutter* 1989). Studies have shown that ammonia can cause alterations in the respiratory tract and thus may interfere with the mucociliary clearance or alveolar phagocytosis and facilitate further colonisation and damage by pathogenic bacteria such as *P. multocida* (*Robinson et al.* 1990, *Done* 1991).

The *P. multocida* toxin (PMT) produced by some strains is known to be of crucial signifi-

cance in the pathogenesis of progressive atrophic rhinitis (*De Jong* 1992, *Chanter & Rutter* 1989), although it may not enhance the pulmonary pathogenicity of *P. multocida* (*Bækbo* 1988). A few hours of exposure to aerial ammonia at concentrations of 50 and 100 ppm reduces the systemic and local resistance to infection with *P. multocida* type A in the lungs of nursing piglets (*Neumann et al.* 1987).

The objective of the experimental study reported here was to examine whether aerial ammonia alone could predispose the respiratory system to infection with toxigenic *P. multocida* type A. Pigs were exposed to ammonia for the duration of the grower-fattening period. This is known to be the period where natural infection is most common (*Pijoan* 1992).

Materials and methods

Experimental design

The experiment included 10 Landrace X Large White pigs obtained from a specific-pathogen-free herd free from *Mycoplasma hyopneumoniae,* toxigenic strains of *P. multocida* and *Actinobacillus pleuropneumoniae.* On arrival at the experimental facilities, the pigs were weighed (average weight: 24 kg), dewormed, bled, marked with individual ear tags, and nasal swabs were taken. The animals were randomly assigned to 2 groups of 5 isolated from each other in 2 ammonia-exposure rooms. Following a 17-day adjustment period, the ammonia-exposure regimens were started and continued until the pigs were euthanized 59 days later. The test group was continuously exposed to 50 ppm $\mp$ 10 ppm of aerial ammonia while the control group only experienced naturally occurring ammonia (below 5 ppm). Ten, 21, 35 and 49 days after the onset of ammonia exposure, all pigs were exposed to an aerosol of toxigenic *P. multocida* type A.

Every week, pigs were bled and every second week their body weights were recorded and nasal swabs taken. Clinical signs of disease were recorded and the rectal temperature was measured when indicated. Feed consumption was recorded daily in each room. All pigs were fed *ad lib.* with a fortified pelleted corn/soybean-meal formula containing 19% crude protein with no antibiotics added.

One week after the last aerosol exposure, the pigs were killed and subjected to gross examination by necropsy. Samples were collected from the lung (areas of consolidation if any, otherwise from the ventral part of the cranial and middle lobe), kidney, conchae (swab) and tonsils.

Ammonia exposure

The 2 ammonia-exposure rooms were identical, each with a 9.9 m^2 solid concrete floor with straw bedding. Positive-pressure fans ventilated the rooms and the temperature was controlled by thermostat (19 °C $\mp$ 4 °C). Ammonia (99.2% purity) was administered as a compressed gas from a cylinder. The supply to the test units was controlled by automatic gas monitors (Gastech model 4440; GasTech Inc., Holland) connected to pilot valves (Type CVK, Danfoss, Denmark), which were attached to the gas cylinders. The gas detector was placed on the wall at a position, which, by measurements with gas detection tubes (Dräger Teknik A/S, Denmark), was representative for the concentration at pig level.

The concentration of ammonia measured by the gas monitors was continuously recorded and stored by a data recorder (Miniscript K, Metrawatt GMBH, Germany). To ensure that the ammonia was distributed evenly in the rooms, fans were placed in the ceiling and behind the gas cylinders. The concentration of ammonia was double-checked regularly with gas detection tubes.

Temperature and relative humidity were measured continuously by a thermohydrograph.

Bacterial inoculum

For the aerosol a lyophilized strain of toxigenic *P. multocida* type A (strain number PB1), originally isolated from the lungs of a pig with pneumonia (*Bækbo* 1986), was reconstituted and passaged twice on solid bovine blood agar plates (37 °C for 24 h). The bacterial culture was washed from the plates with sterile saline solution. Using 10-fold dilutions the bacterial concentration was measured to be 1.2×10^8, 2.7×10^8, 7.5×10^8 and 11.0×10^8 cfu/ml, respectively, at the 4 times of exposure.

Exposure of pigs to Aerosolized microorganisms

The aerosol chamber consisted of a wooden box (200×90×91 cm). Admittance to the box was through a gate in the side. Pigs were aerosolized in groups of 5 and placed in the chamber immediately before the start of inoculation. The bacterial suspension was transferred to a Collison nebulizer (BGI INCORPORATED, Mass.; particle size 1.8 μm). Compressed air (3.5 Barr) generated by a compressor (Air 50, ARDUA, Denmark) was used to aerosolize the suspension after having been filtered through a microfilter and an active carbon filter. The aerosol was released into the chamber through an opening in the side. In the ceiling of the chamber a hepafilter was connected to a fan pump for ventilation.

To generate the *P. multocida* aerosols the nebuliser was operated for 20 min, during which approximately 12 ml of the bacterial suspension were aerosolized. After the nebulizer was stopped, the pigs were retained in the chamber for 5 more min, during which the bacterial content of the chamber air was reduced by ventilation.

Fifteen min after the start of exposure, a 2-stage Andersen air sampler (*Gillespie et al.* 1981) was connected to an opening in the floor of the chamber. The sampler was operated for 4 sec, corresponding to 1.5 liters of sampled air. Bovine blood agar plates with addition of bacitracin (3.5 μg/ml) and neomycin sulphate (2.0 μg/ml) were used in the air sampler (*Nielsen et al.* 1991).

Serological assays and bacteriological examinations

Blood samples were drawn from the anterior vena cava by venipuncture into test tubes containing a clotting factor. Serum was harvested by centrifugation at 1200 G for 5 min and stored at –18 °C until testing for the presence of antibodies to PMT and *M. hyopneumoniae.*

The samples were analysed for anti-PMT and anti-*M. hyopneumoniae* antibodies by enzyme-linked immunosorbent assays (ELISA) (*Foged et al.* 1989, *Feld et al.* 1992). Samples were considered positive if the binding of antibodies was inhibited by 50 per cent or more.

Nasal swabs and biopsies from the lung, kidneys and tonsils were cultured for *B. bronchiseptica* and *P. multocida* and tested for PMT as previously described (*Nielsen et al.* 1991). Agar plates from the Andersen sampler were incubated for 24 h at 37 °C and the number of *P. multocida* colonies was counted.

Statistical analysis

Crude relative risks with 95% confidence intervals were estimated (*Dean et al.* 1995) to measure the strength of association between exposure to ammonia and the frequency of reisolation of *P. multocida* from nasal swabs, tonsils and lungs, respectively. The average daily weight gain (ADG) was estimated from the day of launching the ammonia exposure (avg. weight: 37 kg) until the day of slaughter (avg. weight: 90 kg). The differences in weight gain between exposure groups were tested in an analysis of variance (*Statistical Analysis Systems Institute* 1990). The level of significance was set at a value of $p = 0.05$.

Results

The average relative humidity (RR) in the compartments was 75% (min.: 65% RR; max.: 85% RR). The average temperature was 20 °C (min: 18 °C; max: 21 °C).

None of the pigs showed clinical signs of pneumonia during the experiment. Three to 4 days after the start of ammonia exposure, exposed pigs showed minor conjunctival irritation including excessive lacrimation. Sneezing was rarely observed in any of the groups, and none of the pigs developed visible distortion of the snout.

At the start of the experiment, all pigs were found seronegative to PMT, and through the experiment all sera were found negative to *M. hyopneumoniae.* One control pig seroconverted to PMT after the last aerosol exposure, and one test pig had OD values very close to the cut-off. None of the pigs had gross lesions in the lungs at necropsy.

Samples taken during the aerosol exposure periods showed a bacterial concentration of 10^5 colony-forming units per m^3 in the aerosol-exposure chamber.

Results from the bacteriological examination for toxigenic *P. multocida* from nasal swabs collected during experiment, kidney, lung, tonsils and conchae (post mortem) are given in Table 1. The chance of recovering toxigenic *P. multocida* from the third and fourth nasal swabs, respectively, was 2 and 4 times greater in pigs exposed to 50 ppm ammonia compared to the control group. Nasal swabs were examined for the presence of *B. bronchiseptica* at necropsy and all 10 pigs were found positive.

The average daily weight gain was 946 $\mp$ 161 g/day (control group) and 869 g/day $\mp$ 130 g/day (test group). The feed conversion was 476 g/kg feed (control group) and 385 g/kg feed (test group). There was no significant difference in the daily weight gain ($p = 0.41$) of the 2 groups.

Discussion

The concentrations of ammonia used in this study exceeded those normally found in Danish swine herds. Thus, in the finishing units of Danish farms mean concentrations of ammonia of 9 to 14 ppm have been detected (*Bækbo* 1990, *Pedersen et al.* 1996). Likewise, the concentration of toxigenic *P. multocida* in the aerosol chamber was measured to be 10^5 CFU/m^3, which is much higher than the maximum of 48 CFU/m^3 found in the fattening units of 44 Danish swine herds (*Bækbo & Nielsen* 1988).

In our study aerial concentrations of 50 ppm ammonia alone did not predispose growing pigs for lung infection with *P. multocida.* None of the test pigs showed symptoms of pneumonia and, at necropsy, no macroscopic pneumonic lesions could be detected, nor could *P. multocida* be cultured from the lungs of any of the pigs. This is inconsistent with an earlier study indicating that few h of exposure to ammonia levels of 50 and 100 ppm reduce the systemic and local resistance to infection with *P. multocida* in the lungs of unweaned piglets (*Neumann et al.* 1987). However, the use of different age groups of pigs and a different strain of *P. multocida* may account for the observed differences.

The exposure to 50 ppm ammonia appeared to increase the recovery of toxigenic *P. multocida* from nasal swabs taken after the last 2 inoculations. Because the number of pigs included in our study was small, the increased recovery was not statistically significant, and the results should be interpreted with caution. *Hamilton et al.* (1996) found that exposure to ammonia caused an increase in the number of *P. multocida* organisms isolated from the nasal epithelium of 6-week-old piglets, reaching a plateau between 10 ppm and 25 ppm but decreasing to almost no effect at 50 ppm. Recent long-term exposure studies have shown an increase in the

Table 1. Recovery of toxigenic *Pasteurelle multocida* from nasal swabs, tonsils, lungs and kidneys.

Tissue	Group		cRR	95% CI
	<5 ppm*	50 ppm*		
Nas 1	0/5	0/5	–	–
Nas 2	0/5	2/5 (26, 30)	–	–
Nas 3	1/5 (29)	2/5 (26, 30)	2.00	(0.26-15.62)
Nas 4	1/5 (29)	4/5 (26, 27, 30, 35)	4.00	(0.66-24.37)
Conchae	2/5 (30, 43)	3/5 (26, 35, 39)	1.50	(0.41-5.45)
Tonsil	2/5 (28, 43)	3/5 (26, 27, 35)	1.50	(0.41-5.45)
Lung	0/5	0/5	–	–
Kidney	0/5	0/5	–	–

Nas 1= Nasal swab, arrival.
Nas 2, 3, 4 = Nasal swabs 1 week following *Pasteurella multocida* inoculation.
cRR = Crude Relative Risk.
CI = Confidence Interval.
* Data are expressed as No. of positive samples/No. of samples.
Brackets = The identification-number of those pigs from which toxigenic *P. multocida* could be isolated.

colonization by *P. multocida* on the nasal turbinate at 20 ppm of aerial ammonia (*Hamilton et al.* 1998).

In our study the mean daily weight gain was 80 grams higher among non-exposed pigs compared to the pigs exposed to ammonia. This difference in growth performance was not statistically significant. Due to the shared feeding of pigs, which gives high standard deviations for daily weight gain, and due to the low number of observations a statistical difference could not be expected within the study. *Curtis et al.* (1975) found no effect on the gain of body weight in healthy, weaned pigs exposed to 50 ppm ammonia for a period up to 40 days.

The number of viable *P. multocida* recovered from the air in the aerosol chamber was much lower than the number present in the suspension used for nebulizing. A similar finding was reported by *Jacobsen et al.* (1996) who used a similar aerosol chamber for virulence studies of *A. pleuropneumoniae.* The decrease may both be due to condensation of aerosol on the inner surfaces of the aerosol chamber, factors reducing the viability of the infective organisms (*Thomson et al.* 1992) and due to inhalation by the exposed pigs.

Since the results from this study suggest that ammonia alone is unlikely to predispose the lungs to infection with *P. multocida,* further investigations should focus on the relevance of the combined effect of ammonia, *P. multocida* and *M. hyopneumoniae* in the pathogenesis of pneumonia. *M. hyopneumoniae* is well-known to predispose the lungs to infection with *P. multocida* (*Ciprián et al.* 1994).

Acknowledgements

This experiment was supported by Novo Fonden, Bernhard Bangs Mindefond, Forsøgsleder R. Nørtoft Thomsens legat til fremme af dansk husdyrbrugsforskning, Astrid og H. Zehngraffs Legat, Upjohns Jordbrugsvidenskabelige Fond. The authors thank the Department of Clinical Studies, Royal Veterinary and Agricultural University, for the housing of pigs and technical assistance. Thanks are due to professor Knud Nielsen for valuable discussion.

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Sammendrag

Eksperimentelt infektionsforsøg til belysning af om aerogen ammoniak alene kan prædisponere slagtesvin for luftvejsinfektion med toxinproducerende Pasteurella multocida.

Formålet med det beskrevne eksperimentelle forsøg var at undersøge, om eksponering for 50 ppm ammoniak kan disponere slagtesvin for lungeinfektion med *Pasteurella multocida.* To grupper à 5 grise blev i 59 dage (fra 37 kg til 90 kg legemsvægt) eksponeret kontinuerligt for henholdsvis 50 ppm ammoniak og mindre end 5 ppm ammoniak (kontrolgruppe).Ti, 21, 35 og 49 dage efter starten på ammoniak eksponering blev grisene i et specielt designet aerosolkammer udsat for en aerosol (gennemsnitlig aerosolkoncentration: 10^5 CFU toxinproducerende *P. multocida* type A per m^3). Hver anden uge blev grisene vejet, og næsesvabre udtaget. Grisene blev aflivet og obduceret en uge efter den sidste aerosolisering.

Ingen af grisene udviste kliniske symptomer på pneumoni eller nysesyge. Ved obduktion var alle grise uden pneumoniske forandringer, og toxinproducerende *P. multocida* kunne ikke isoleres fra lungerne. Sammenlignet med kontrolgruppen var der mod slutningen af forsøget iblandt forsøgsgrisene en øget chance for at isolere toxinproducerende *P. multocida* fra næsesvabre. Den daglige tilvækst var i forsøgsgruppen lavere end i kontrolgruppen.

Undersøgelsen konkluderer, at eksponering for 50 ppm ammoniak formodentlig ikke kan prædisponere ung- og slagtesvin for lungeinfektion med toxinproducerende *P. multocida.*

(Received March 17, 1999; accepted March 18, 1999).

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