Non Invasive (CT) Investigation of the Lung in *Bordetella bronchiseptica* Infected Pigs

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Summary

Bordetella bronchiseptica produced pneumonia was studied in young piglets. At the beginning of the experiment, 30 artificially reared 3-day-old piglets were divided into two groups: group A – uninfected piglets, control group (n=10) and group B – piglets infected with *B bronchiseptica,* experimental group (n=20). The *B. bronchiseptica* infection (10^6 CFU/ml) was performed on day 4. In Group B, clinical signs including mild serous nasal discharge, sneezing, panting, and hoarseness appeared from day 4. Computed tomography (CT) performed on day 16 demonstrated lung lesions attributable to colonisation by *B. bronchiseptica* in the infected pigs. The gross pathological findings confirmed the results obtained by CT.

Key words

Bordetella bronchiseptica, Porcine respiratory disease complex, Computed tomography

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Aim

Respiratory disease is one of the most important health concerns for modern swine production. The term porcine respiratory disease complex (PRDC) refers to a condition in which an interaction between various pathogens and inappropriate environmental conditions lead to severe respiratory disorders. Disease entities occurring in the simultaneous presence of multiple pathogens coupled with environmental predisposing factors are common in the practice and they have enormous importance for the profitability of production.

PRDC primary pathogens can be viruses or bacteria, while the secondary pathogens are mostly bacteria (Brockmeier et al., 2002a). *B. bronchiseptica* is frequently isolated from respiratory conditions produced by multiple aetiological factors. Its dermonecrotic toxin (DNT) has a fundamental role in producing respiratory disease in swine (Brockmeier et al., 2002b). Previous studies suggest that the concurrent presence of *B. bronchiseptica* and other respiratory pathogens develops more severe disease than that produced by infection with *B. bronchiseptica* alone (Brockmeier et al., 2000; Brockmeier, 2004; Brockmeier et al., 2008). *B. bronchiseptica* and toxigenic *P. multocida* are known to work together in producing the progressive form of porcine atrophic rhinitis (Chanter et al., 1989). *B. bronchiseptica* has also been demonstrated to be able to produce pneumonia in young piglets (Underdahl et al., 1982).

In the present study we examined the *B. bronchiseptica* produced pneumonia in young piglets. Computed tomography (CT) was applied to follow up the pathological events in the lung.

Material and methods

Thirty 3-day-old pigs were used in the study. The piglets included in the experiment originated from a herd of high health status, in which the incidence of respiratory diseases was negligible. The sows were free from toxigenic *B. bronchiseptica*. On the 3rd day of life, a total of 30 female piglets were selected, and transported to the experimental animal facility (day 0 of the experiment). After an early weaning, the piglets were artificially reared with milk-replacer until day 16 and then with solid feed until the termination of the experiment (day 39). On the day of their arrival, the piglets were placed into battery cages in two rooms. Two groups were assigned separately in two rooms: group A – uninfected piglets, control group (n=10) and group B – piglets infected with *B bronchiseptica,* experimental group (n=20). Air temperature was adjusted to 27°C. The cages and the rooms were cleaned twice a day, and the piglet-rearing equipment was cleaned every second day. Animal tenders wore protective clothing, and disinfected their hands and feet with the aqueous solution of Virkon S® (Antec, Novo Mesto, Croatia) when entering the rooms. Up to day 16, the piglets were fed a milk replacer diet consisting of skim milk powder, vegetable fats and whey powder, containing 23% crude protein, 23% ether extract and 1.6% lysine (Salvana Ferkel Ammen Milch®, Salvana Tiernahrung, Sparrieshoop, Germany) from 'Mambo' automatic feeder (Sloten, Deventer, The Netherlands). From day 7, a dry coarse meal containing 16 MJ/kg metabolizable energy, 18.5% crude protein, 9% ether extract and 1.65% lysine (Salvana Premeal®, Salvana Tiernahrung, Sparrieshoop, Germany) was also given to the piglets *ad libitum*, and then from day 16 up to the end of the experiment (day 39) only this latter diet was available to them. Drinking water was provided from nipple drinkers, and initially this was complemented with water offered from plastics drinking bowls of free water surface.

Group B piglets were infected with *B. bronchiseptica* (strain KM22, dose: 10^6 CFU/mL) on day 4. The bacterial suspensions were prepared as described previously (Magyar et al., 2002). A volume of 0.5 mL was inoculated through an endotracheal tube in all cases.

The clinical signs were recorded daily during the experiment. The piglets were weighed on days 4, 16, 25 and 39.

CT examinations were performed on days 4, 16, 25 and 39 to detect lesions in the lung. Combinations of the following active ingredients were used for premedication: azaperone (Stresnil®, Janssen, Beerse, Belgium, 4 mg/bwkg, i.m.), ketamine (CP-Ketamin 10%®, CP-Pharma, Burgdorf, Germany, 10 mg/bwkg i.m.), xylazine (CP-Xylazine 2%®, CP Pharma, Burgdorf, Germany, 1 mg/bwkg, i.m.), atropine (Atropinum sulphuricum 0.1%®, EGIS, Budapest, Hungary, 0.04 mg/bwkg, i.m.). After premedication, a balloon-type endotracheal tube was introduced into the trachea, and then anaesthesia was induced by the inhalation of isoflurane (Forane®, Abbott, Illinois, USA) in a mixture with 2% (v/v) oxygen. The animals were placed in supine position on a special supporting structure. To make CT scans, artificial breath-holding was applied during the thoracic scan. CT scans of the entire volume of the lungs were made with a SIEMENS Somatom Emotion 6 multislice CT scanner (Siemens, Erlangen, Germany); tube voltage: 130 kV, dose 100 mAs, FoV 200 mm). From the collected data cross-sectional images of 2- and 5-mm slice thickness were reconstructed, with full overlapping. The images were analysed using the Medical Image Processing (2006) software.

At termination, the pigs were humanly killed and lung lesions were examined post mortem. Post mortem examinations were performed on day 39. For histopathological examination, samples were taken from lung areas showing pathological changes. Tissue samples were fixed in 4% formalin solution, embedded in paraffin, sectioned, and the sections were stained with haematoxylin and eosin (HE).

Results and discussion

Piglets of Group A did not show clinical signs during the experiment. In Group B clinical signs including a mild serous nasal discharge, sneezing, panting and hoarseness appeared from 4 days after *B. bronchiseptica* infection. There were no significant difference between the groups in the growth rate of piglets ($P>0.05$). No lesions were seen in Group A at any of the test dates (Table 1).

On day 16, 25 and 39 lung lesions were seen in 19 out of the 20 piglets of Group B, which were characterised by a mild to moderate density increase (around –600/–300 on the Hounsfield scale, HU), as compared to the normal density in the pneumatised parenchymal areas of the lung (which is around –700/–800 HU). This density increase was the result of an inflammatory process (exudate formation, cell proliferation) (Figure 1).

Table 1. Number of piglets showing pathological lung lesions based upon the CT scan and the gross pathological examination performed at the end of the experiment as related to the total number of animals in the group

Group	Day of the experiment				Necropsy
	Day 4	Day 16	Day 25	Day 39	
A	0/10	0/10	0/10	0/10	0/10
B	0/20	19/20	19/20	19/20	19/20

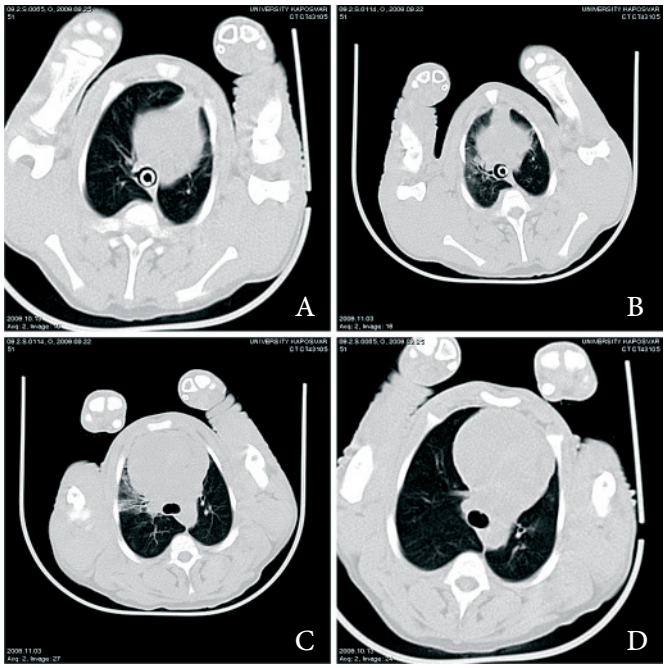

Figure 1. The affected areas in the lung of a *B. bronchiseptica* infected piglet with density increase on the CT scans (on day16 and 39); A – anterior lobes on day 16, B – anterior lobes on day 39, C – intermediate lobes on day 16, D – intermediate lobes on day 39

In Group A none of the piglets had changes in the lung while the lungs of 19 out of the 20 surviving animals of Group B showed pathological lesions. These lesions were located mainly in the anterior and intermediate lobes and in the cranial third of the posterior lobe, and their size ranged from lesions involving a few lobules to changes extending to the entire lobe (Figure 2).

The lesions occurred mainly in the form of acute catarrhal pneumonia (Figure 2) with chronic catarrhal areas, hemorrhagia, pleuritis and fibrosis. Some animals developed combination of catarrhal and purulent or catarrhal and fibrinous pneumonia.

Conclusions

The *B. bronchiseptica* mono-infection was able to produce lung lesions in young pigs. Our results also indicate that CT can be applied for studying the pathological conditions in the lower respiratory tract of swine. Valuable information can be collected about the formation of the lesions over time as well as about the nature of the changes in the lung tissues.

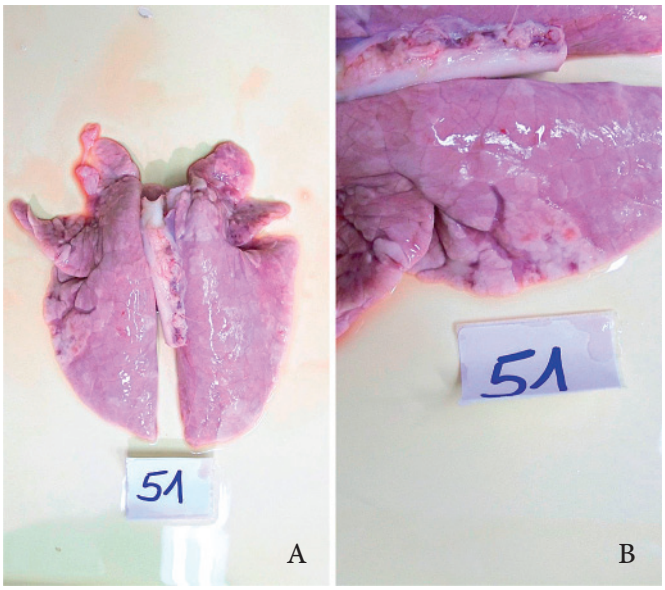

Figure 2. The location of the lung lesions of a *B. bronchiseptica* infected piglet (anterior, intermediate lobes and in the cranial third of the posterior lobe)

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