Sensitisation against environmental antigens and respiratory symptoms in swine workers

MARJA-L KATILA, R A MÄNTYJÄRVI, AND TARJA H OJANEN

From the Department of Clinical Microbiology, University of Kuopio, Finland

ABSTRACT Adverse effects caused by airborne material to the respiratory tract are due either to non-specific irritation or to hypersensitivity. In this study 20 people employed in swine barns and 18 controls were tested for sensitisation against dusts present in the barns. Immunoprecipitation and enzyme-linked immunoassay (ELISA) were used to test for IgG antibodies; IgE antibodies against swine epithelium were tested using solid phase radioimmunoassay. Precipitins against swine antigens were found in two swine workers; while ELISA found six to be sensitised. Sensitisation against swine antigens correlated with exposure but not with the presence of symptoms. No IgE antibodies were found. Precipitins against feed antigens were detected in 12 workers; in nine of the 12 with symptoms, and in three of the eight asymptomatic workers. No single antigen was of special importance as an inducer of sensitisation. Sensitisation against feed dusts in barns, as indicated by the presence of circulating antibodies, suggests an immunological background for persistent symptoms. A large antigen panel should be used in testing for sensitisation because of the many immunogenic dusts present in the air in swine barns.

The atmosphere of swine barns contains numerous airborne materials that are potentially hazardous to the respiratory tract by reason of their large quantity or of their qualitative nature. The total dust load is known to reach temporarily very high levels during feeding.^{1,2} According to Nilsson,² pig feeds and particles of animal origin are quantitatively the most important components of the particulate matter in the air. In addition, chemicals such as ammonia and hydrogen sulphide, also of animal origin, are present in gaseous form in barn atmospheres.³ The detrimental effect of dusts and other aerial impurities on pork production has been recognised in recent years³ and has become a factor of increasing importance with the trend toward bigger units in swine confinement systems. Airborne biological materials also have an adverse effect on the respiratory tract of anyone working in the dusty barn environment and respiratory symptoms are a common problem among people employed in swine production.²

An earlier survey on the prevalence of farmer's lung⁴ found several workers with respiratory disorders associated with working in swine barns; the

present report describes a study performed to detect the occurrence of sensitisation against pig-derived antigens and against commercial pig feeds in swine workers.

Material

Seventeen non-smoking farmers (mean age 41 years), occupied either full-time or part-time in swine production, took part in the study. They were randomly selected from the non-smoking swine producing participants in an earlier survey.⁴ Each had been interviewed for respiratory symptoms and was regarded as symptomatic if there had been cough and phlegm production on most days for a minimum of three months a year, or attacks of breathlessness associated with the working environment, or both. Nine were symptomatic and eight asymptomatic on these criteria.

Three swine workers (coded later by initials) were also included in the series, although they were not from the previous survey. They all had respiratory symptoms for which they had sought medical advice. All had an occupational history of 5-10 years in swine production and had earlier been engaged in other agricultural work without noticeable difficulty.

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Their ages ranged from 40 to 56 at the time the symptoms appeared.

A group of rural dwellers (mean age 53) with no exposure to agricultural dusts either in their present occupation or for a minimum of four years was used as a control group.

Methods

PREPARATION OF ANTIGENS FOR THE SEROLOGICAL TESTS

Swine serum was used in the testing undiluted. A swine epithelial antigen was prepared by suspending skin scrapings in 0.9% saline followed by disruption by ultrasonic treatment (MSE 1174 MK2, Crawley, UK). Centrifugation at 1000 g for 15 min yielded a supernatant with a protein concentration of 4 mg/ml, which was used as the test antigen.

Antigens were prepared from three commercial pig feeds and from a commercial feed component, "peciloprotein," which is added into feeds as a protein source. Feed antigens were made by suspending the commercial product in phosphate buffered saline pH 7.0 (PBS). The suspension was mechanically disrupted first with Ultra Turrax (Janke and Kunkel, Staufen i Breisgau, FRG) and then for 2 min with a cell homogeniser (MSK, B Braun Melsungen, FRG). The supernatant achieved after centrifugation at 1000 g for 15 min was used as the antigen; the protein concentration of this antigen was 10-20 mg/ml.

An important source of protein used in commercial feeds as an alternative to peciloprotein is the yeast protein derived from *Candida utilis*, also called *C. carborea*. An antigen of *C. utilis* (VTT-C-71015) and another of *C. albicans* (VTT-C-75003), a common environmental yeast, were made using microbe strains obtained from the State Technical Research Centre of Finland. The microbes were grown in synthetic medium VII⁵ for three days at 37°C. Extracts of washed yeast cells were prepared by the procedure described above. The protein concentration of the final antigen was 10 mg/ml.

Aspergillus fumigatus antigen was purchased from Bencard (Bencard, Brentford, England) and those of the thermophilic actinomycetes, *Micropolyspora faeni* and *Thermoactinomyces vulgaris*, were provided by the Mycological Reference Laboratory, London, England. Each was used according to the instructions of the makers.

ANTIBODY TESTS

Precipitating antibodies were shown by a double diffusion micromethod described previously.⁶ The enzyme-linked immunosorbent assay (ELISA), also

used to detect antibodies of the IgG class, was performed in microtitre plates using the method of Voller *et al*⁷ with minor modifications.⁸ The results were expressed as absorbances at a serum dilution of 1:100 measured by a Titertek Multiscan spectrophotometer (Flow Laboratories, Eflab, Helsinki, Finland).

A solid phase radioimmunoassay (RIA) was used to test sera for antibodies of the IgE class against swine epithelium. Polystyrene balls (Precision Plastic Ball Co, Chicago, Ill) were used as carriers. The conjugate was ¹²⁵I-labelled antihuman IgE purchased from Pharmacia, Sweden. The procedure was similar to that of ELISA except that sera were tested undiluted. The conjugate was used as instructed by the manufacturer.

Results

Table 1 shows the symptoms noted in the swine workers. Dyspnoea with or without wheezing was the most common complaint. All three workers not in the original survey had a cough with periods of daily phlegm production and attacks of breathlessness with occasional wheezing associated with the working environment. Bronchial hyperreactivity on methacholine challenge had been recognised in two of them, but the clinical picture during a follow-up was that of chronic bronchitis. Cough and dyspnoea appeared while working in the swine barn. Each also had a history suggesting a dual type reaction with another aggravation of symptoms after several hours. Malaise and febrile reactions were also complained of by two of the three after work in the swine barn. Each had been in hospital at some stage in their disease, but no signs of allergic alveolitis had been found.

Table 1 also shows the presence of antibodies against various swine barn associated antigens in the swine workers with respiratory symptoms. Positive reactions were obtained with all antigens. Only three of the 12 did not have antibodies against any of the antigens used.

Table 2 summarises the antibody findings in swine workers with or without symptoms and in the control group. Precipitating antibodies against swine serum and epithelium were shown in the sera of two of the 20 swine workers whereas all the controls were negative. Lines of identity were formed between the two swine antigen preparations, but unidentical precipitates were also present, with the epithelial antigen giving more lines of precipitation.

When the sera were tested against the swine epithelial antigen by the more sensitive ELISA technique, additional sensitised individuals were found. The sera of the two precipitin-positive people

Table 1 Respiratory symptoms and precipitating antibodies in symptomatic swine workers

Code	Type of symptom	Antibodies against							
		Swine serum	Swine epithelium	Pig feeds	Peciloprotein	C utilis	A fumigatus	M faeni	T vulgaris
013	Br	+	+	+	-	-	-	+	+
283	Br	-	-	+	-	-	-	-	-
007	Br	-	-	-	+	+	-	-	-
317	Br + D	-	-	-	-	+	-	+	+
NJ	Br + DW	-	-	+	-	-	+	-	-
RM	Br + DW	-	-	+	+	-	-	-	-
008	DW	-	-	+	-	-	-	-	-
045	D	-	-	+	+	-	-	-	-
033	C + D	-	-	+	-	-	-	-	-
205	C + D	-	-	-	-	-	-	-	-
KK	C + DW	-	-	-	-	-	-	-	-
014	DW	-	-	-	-	-	-	-	-

C = Non-productive cough.

Br = Cough and phlegm 3 m/yr.

D = Attacks of dyspnoea.

DW = Dyspnoea with wheezing.

Table 2 Precipitating antibodies against various environmental antigens

		Antibodies against						
		Swine	Pig feeds	Peciloprotein	C utilis	C albicans	A fumigatus	Termophilic actinomycetes
No of swine workers								
With symptoms	12	1	7	3	2	1	1	2
Without symptoms	8	1	2	1	0	2	1	0
Total	20	2	9	4	2	3	2	2
Controls	18	0	3	2	2	3	1	4

Fig 1 Distribution of ELISA absorbances in IgG ELISA against pig epithelium in swine workers and controls. Sera were tested at a dilution of 1/100. Each point represents one test serum.

showed the highest absorbances in the IgG-ELISA (fig 1). The distribution of the ELISA absorbances in the swine workers had a wide range. By contrast, the controls had low ELISA absorbances (mean 0.192 ± 0.054 SD, fig 1) with one exception, a female pharmacist aged 48, who was excluded from the calculation of the mean. Subsequent inquiry showed that she had been employed in pig breeding at her home farm 30 years earlier, with occasional later exposures during summer vacations.

Though the degree of sensitisation correlated with the antigen exposure, it did not correlate with the presence of respiratory symptoms, both symptomatic and asymptomatic individuals having high and low absorbances. Six of the 20 swine workers showed a sensitisation of some degree, as indicated by an absorbance above 0.3 (the negative mean ± 2 SD). The limit for a positive precipitin test seemed to lie between absorbances of 0.6 and 0.7.

Solid phase RIA test was also performed to detect the degree of sensitisation by IgE class antibodies against the swine epithelial antigen. None of the symptomatic or asymptomatic swine workers showed measurable amounts of specific antibodies of the IgE class. A serum containing IgE antibodies against rat epithelium, prepared identically to that of

Fig 2 Demonstration of specificity and non-specificity of precipitates between pig feed antigen (Ag) and patient sera. Serum A formed true antigen-antibody precipitates with pig feed antigen shown by three lines showing a reaction of identity when tested in two neighbouring wells. Sera B and C, on the contrary, showed reactions of unidentity representing non-specific precipitates.

pig epithelium, was used as a positive control.

Precipitating antibodies against one or more of the three pig feeds were shown in nine of the 20 swine workers (table 2). All the precipitates were confirmed to represent true antigen-antibody reactions by the formation of lines of identity when the same serum was tested in two neighbouring wells (fig 2). Antibodies against peciloprotein were found in four and against *C utilis* in two swine workers (table 2). Precipitating antibodies against feed associated antigens were more common among the symptomatic (9/12) than asymptomatic (3/8) individuals. Precipitins were also found in three without recent occupational or other identifiable exposure to pig feeds.

Precipitating antibodies against *C utilis* and *C albicans* were shown equally often in the exposed and unexposed groups (table 2). No cross-reactivity was seen between the two yeasts since precipitins against the two yeasts were never found in the same individual.

The sera were also tested for antibodies against the microbes associated with farmer's lung (table 2). Precipitating antibodies against *A fumigatus* were present in two swine workers and in one control, and those against *M faeni* and *T vulgaris* in two symptomatic swine workers. Four of the controls were also precipitin positive against *T vulgaris*.

Discussion

The atmosphere in swine barns presents many undesirable agents to the respiratory tracts of those who work there. The dust load, which may be very great during feeding times,^{1,2} has mechanically irritating and biological effects. Many immunologically active materials are also present in this environment. Dusty material originating both from commercial and domestic feeds, disrupting and decomposing vegetable matter with possible microbial antigens, as well as animal proteins excreted by and scaled off the swine, are all agents with a potential immunogenic capacity. Gaseous materials, especially ammonia and hydrogen sulphide, introduced into the atmosphere from urine and faeces, may also play a part as chemical irritants and possible adjuvant agents to the biological dusts.

The dusts from feeds have in most cases been the target when the atmosphere in swine barns has been examined. The work of Nilsson² showed, however, that the dust caused by the animals themselves is also a quantitatively important portion of the dust.

Respiratory symptoms arising in swine barn employees are diverse and the causative agents often hard to identify. In this study those with swine barn exposure were studied serologically. To exclude the effects of smoking on respiratory symptoms, a group of non-smokers was selected for the study.

The results showed that the occupational exposure had induced IgG class antibodies against swine epithelial and serum antigens in about a third of those exposed. The presence of antibodies had, however, no correlation with the presence of symptoms. The maintenance of a state of sensitisation for 30 years with only short occasional re-exposures was apparent in one of the controls.

The tests for antibodies of the IgE class against swine epithelium in those exposed were all negative. In a group of 20 one would have expected to find an atopic individual prone to form IgE response. Possibly, however, atopic individuals avoid a dusty working environment of this type due to easily induced symptoms.

Precipitating antibodies against the feed associated antigens were more common among symptomatic than asymptomatic workers, which suggests that the feed antigens may induce the respiratory symptoms. The reasons for the presence of antibodies against feed antigens in occupationally unexposed individuals remains unclear. The controls were rural inhabitants, and some of them had a distant history of work on farms, mostly dating from their adolescence. The commercial feeds are, on the other hand, very complex mixtures of potentially immuno-

genic proteins from different sources, including fish and soy bean flour as well as animal blood products.

Yeast proteins did not stand out as important inducers of sensitisation. Being natural environmental microbes, the yeasts had caused sensitisation among the controls as often as they did among the swine worker group. Sensitisation against the thermophilic actinomycetes was as common in both groups, as could be expected from earlier findings.⁶

This study shows that individuals exposed to swine work are sensitised to many of the agents present in their working environment. Sensitisation itself, however, is not a sign of disease. In those with persistent respiratory symptoms, IgG class antibodies may be used as corroborative evidence of the immunological aetiology of an occupation-associated disease. In testing, however, a large panel of environmental antigens other than those included in this study⁹ should be used, since this study did not implicate any single antigen to be of special importance as an inducer of IgG antibodies in swine workers. Though our limited IgE tests remained negative, atopic individuals should also be tested for IgE antibodies using a similar antigen panel.

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