

Immune changes in animal breeders: a pilot study conducted in northern Italy

Ramin Tabibi¹, Emanuela Corsini², Gabri Brambilla¹, Luigi Bonizzi³, Gianlodovico Melzi d'Eril⁴, Giulia Rabozzi¹, Maryam Sokooti¹, Luisa Romanò⁵, Chiara Somaruga¹, Francesca Vellere¹, Alessandro Zanetti⁵, Claudio Colosio¹

¹ Department of Occupational and Environmental Health, University of Milan, International Centre for Rural Health, University Hospital San Paolo, Milan, Italy

² Laboratory of Toxicology, Department of Pharmacological Sciences, University of Milan, Italy

³ Department of Animal Pathology, Hygiene and Veterinary Public Health, University of Milan, Italy

⁴ Department of Medicine, Surgery and Dentistry, University of Milan, Italy

⁵ Department of Public Health – Microbiology – Virology, University of Milan, Italy

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Abstract

Objective: Farming is associated with exposure to a wide variety of risk factors including organic dusts, endotoxins, allergens and other chemicals. The ability of some of these agents to interact with the immune system is demonstrated in the presented study which was undertaken to evaluate the relationship between pig and cow breeding, and the immune system early changes. Particular attention is paid to selected serum cytokines.

Methods: Sixty four animal breeders (36 cattle and 28 pig breeders) were selected as the exposed group, and 32 rural workers not engaged in animal breeding were utilised as the controls. Personal data were collected through a questionnaire, and selected serum parameters measured, including cytokines IL-6, IL-8, IL-10, IFN γ and TNF α , immunoglobulins and proteins, and total and differential white blood cell counts.

Results: The study stresses the significant increase of TNF- α , IL-8, and IL-10 in animal breeders, with the highest values in pig breeders, and a slight but statistically significant increase in albumin and total serum proteins.

Conclusions: The findings of the presented study suggest a condition of immune system activation in animal breeders, with the highest levels observed in pig breeders. These changes may be attributable to exposure to organic dusts, endotoxins, or to the different biological agents present in the rural environment. The prognostic significance of these findings, however, remains unclear, but the observed changes might be indicative of a risk of developing respiratory toxic and allergic diseases, which need to be further investigated.

Key words

pig breeders, farmers, inflammatory cytokines, interleukins, biological risks, organic dust, endotoxins

INTRODUCTION

Animal breeders are occupationally exposed to numerous agents that are potentially capable of interacting with the immune system. Some of these agents include bacteria such as *Brucella* spp., *Erysipelothrix rhusiopathiae*, *Leptospiriosis* spp., *Mycobacterium* spp., *Streptococcus* spp., as well as viruses, such as *Hepatitis E* and *Influenza* [1, 2, 3, 4, 5, 6, 7, 8, 9]. The contact with these biological agents exposes workers to a risk that is still poorly defined.

In addition to microorganisms, occupational exposure on animal breeding farms might also result in contact with other biological risk factors, in particular organic dusts. In general, the air in livestock buildings contains a large variety of microorganisms, gases, and a considerable amount of dusts which can remain suspended in the air for long periods, and can therefore be inhaled [10, 11]. Dusts are characterized by their heterogeneous composition, containing endotoxin,

lectins, pollens, feeds, and antibiotic residues. There is strong epidemiological evidence that organic dusts and bacteria can cause infectious and allergic diseases, both in animals and farm workers [12]. High exposure to organic dusts, especially when contaminated with large amounts of endotoxin, may result in a flu-like disease, with symptoms such as fever, chills, dry cough, malaise, mild dyspnea, headache and muscle pain, the so-called 'organic dust toxic syndrome' (ODTS) [13, 14]. In subjects without any sign of overt diseases, inhalation of organic dusts might also result in slight changes, indicative of an immune system activation such as increase in blood cell count, IL6 and TNF α observed particularly in winter when ventilation is reduced [16, 17]. It is unclear whether these changes represent an early adverse effect, able to evolve into overt diseases, or only adaptive and transient changes consequent with exposure to immune system activators.

Studies addressing the interactions between the immune system and rural indoor environment, in particular animal breeding farms, are few and far between, and no firm conclusions have been drawn from them [18, 19]. The presented pilot study was carried out with the aim of studying selected immune parameters in a group of animal breeders

Address for correspondence: Ramin Tabibi, Department of Occupational and Environmental Health, University of Milan; International Centre for Rural Health, University Hospital San Paolo, Via S. Vigilio 43, 20142 Milan, Italy.
E-mail: ramin.tabibi@unimi.it

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(exposed), and in agricultural workers not engaged in animal breeding (controls), to evaluate the presence of possible differences between the groups, which suggest the possible presence of job related patterns of activation/deactivation of the immune system.

MATERIALS AND METHODS

Study Subjects. This cross-sectional pilot study was conducted in the region of Lombardy, northern Italy, in small size agricultural enterprises provided with occupational health surveillance at the workplace by the International Centre for Rural Health, hosted by the University Hospital San Paolo, in Milan, Italy, during the period between January 2009 - September 2010. The study was approved by the Ethical Committee of San Paolo Hospital. Before beginning of the study, all workers were informed about the objectives and the methods to be employed, and those who agreed to participate signed an informed consent. A total of 96 subjects were selected (91 males and 5 females), 64 animal breeders (exposed group) and 32 rural workers engaged only in agricultural activities but not in animal breeding (control group). The exposed group included 36 cattle and 28 pig breeders. Criteria for exclusion were intake of medications known to affect the immune system, i.e. steroids and non-steroidal anti-inflammatory drugs, recent vaccinations, or presence of malignancies, inflammations and infections, as well as conditions of immunodeficiency. None of the workers showed any of these conditions when the study was conducted.

Clinical data and personal information collection. Personal data such as socio-demographic and clinical information, personal habits, smoking and alcohol intake, as well as the results of previous physical and laboratory examination were obtained from the personal data collection forms routinely used by our Centre to collect health surveillance individual data sheets.

Blood sample collection. Before physical examination, 10 ml blood samples were obtained through venepuncture. Within 4 hours after collection, the blood samples were processed and isolated serum was frozen at -20°C until analysis. Samples were collected in the morning, before the beginning of the work shift.

Blood analysis. The following parameters were measured: complete and differential blood cells count, serum proteins, including α_1 , α_2 , β and γ -globulins and total serum proteins, and cytokines (IL-6, IL-8, IL-10, TNF- α , IFN- γ). Cytokine production was assessed by ELISA using commercially available kits (Immunotools, Friesoythe, Germany), following the manufacturer's instructions. Results are expressed in pg/ml. The limit of detection was 1 pg/ml for all cytokine assessed.

Blood cell count was measured with an automated haematology analyzer, Sysmex XT-2000i (Dasit, Milan, Italy). Calibration of the instrument was confirmed each day using 2 levels of controls (Sysmex e-Check Xe Haematology Controls for Sysmex x-series Analyzers, Sysmex Italy), according to the manufacturer's recommendations. Repeated analyses of a sample obtained from a healthy donor was used daily to confirm instrument precision. The between-run imprecision (CV%) was $< 6.97\%$ for all parameters.

Serum proteins were measured by semi-automated agarose electrophoresis with the Hydrasys LC automate (Sebia, Florence, Italy). In the staining compartment, staining (4 minutes with amido black), destaining (3 times, for 3 minutes, 2 minutes and 1 minute, respectively), and drying (75°C for 8 minutes) were conducted automatically, and finally the gels were scanned with a Hyrys densitometer (Sebia, Florence, Italy).

Each day, 2 assays with two 2 aliquots of a serum pool were analysed. The serum pool was prepared from patient specimens filtered through $8\ \mu\text{m}$ (pore size) filters. The pool was aliquoted and stored at -20°C until assay. The between-run imprecisions (CV%) were 1.50 %, 5.61 %, 2.95 %, 2.46 % and 2.8 % for the albumin, α_1 -globulin, α_2 -globulin, β -globulin and γ -globulin fractions, respectively.

RESULTS

91 males and 5 females aged between 19-70 years (median 42 years) were enrolled in the study: 64 breeders (28 pig breeders and 36 cattle breeders) and 32 non-breeders. All workers were in good health conditions.

Data regarding demographic and personal information are shown in Tables 1 and 2. Table 1 compares data between breeders and non-breeder workers in the selected enterprises; Table 2 represents data only for breeders (pig and cattle). There was a significant difference for alcohol intake ($p=0.02$,

Table 1. Demographic and personal information of the study groups (breeders/non-breeders)

Workers/Variables	Age (years) Median (Min-Max)	Weight(kg) Median (Min-Max)	Nationality (Eu-non EU)	Smokers (%)	Cigarettes/day Median (Min-Max)	Alcohol consumption (%)	Alcohol units/day (Min-Max)
Breeders (n=64)	44 (19-70)	75 (60-105)	42-22	21.9	17.5 (2-30)	40.6	2 (1-12)
Non-breeders (n=32)	34 (22-62)	76 (52-100)	28-4	34.4	10 (5-25)	65.6	1.5 (1-5)
p-value	<0.01	0.63	0.02	0.22	0.63	0.02	0.21

Table 2. Demographic and personal information of the breeders (pig/cattle breeders)

Workers/Variables	Age (years) Median (Min-Max)	Weight(kg) Median (Min-Max)	Nationality (Eu-non EU)	Smokers (%)	Cigarettes/day Median (Min-Max)	Alcohol consumption (%)	Alcohol units/day (Min-Max)
Pig breeders (n=28)	39 (19-70)	74.5 (60-105)	22-6	25	17.5 (2-25)	42.9	4 (2-12)
Cattle breeders (n=36)	46 (27-66)	75 (60-91)	20-16	19.4	15 (5-30)	38.9	1.5 (1-5)
p-value	0.12	0.94	0.06	0.56	1	0.75	0.10



Mann-Whitney test) between the study groups (breeders/non-breeders), while no significant difference was detected for weight and smoking habits of the participants (Tab. 1).

Table 3 shows the distribution of total white blood cell counts and differences between breeders and non-breeders; Table 4 shows the distribution among cattle and pig breeders. All the parameters were in the range of reference values for all the workers, and no significant difference were found between the groups, with the exception of an increase in the percentage of eosinophils in the cattle breeders.

Table 3. White blood cell count of agricultural workers (breeders/non-breeders)

Variable	All Breeders (n=64)		Non-breeders (n=32)		p-value
	Median	Min-Max	Median	Min-Max	
WBC (10 ³ /μl)	6.65	3.54-11.19	7.0	4.28-11.21	0.94
Neutrophils (%)	56.4	40.1-74.9	58.3	36.5-68.9	0.74
Lymphocyte (%)	33.2	16.4-48.6	30.10	20.5-54.4	0.73
Monocyte (%)	7.9	4.2-13.6	8.00	5-9.8	0.92
Eosinophil (%)	2.3	0-9	1.7	0-11	0.01
Basophil (%)	0.3	0-1.3	0.3	0.1-1.3	0.20

Table 4. White blood cell count of pig and cattle breeders

Variable	Pig Breeders (n = 28)		Cattle Breeders (n=36)		p-value
	Median	Min-Max	Median	Min-Max	
WBC (10 ³ /μl)	6.53	4.65-11.99	6.8	3.54-10.76	0.82
Neutrophils (%)	59.15	42.4-74.9	54.8	40.1-72.5	0.26
Lymphocyte (%)	32.35	16.6-44	34.3	16.4-48.6	0.49
Monocyte (%)	7.95	4.2-13.6	7.9	5.1-12.3	0.64
Eosinophil (%)	2.15	0-8	2.8	0-9	0.02
Basophil (%)	0.25	1-1.20	0.3	0.0-1.3	0.67

Serum protein electrophoresis results between breeders and non-breeders, and between cattle and pig breeders are shown in Tables 5 and 6, respectively. The results indicated slightly higher values of serum proteins and globulins in animal breeders, compared with the control group. Furthermore, there was a statistically significant difference between occupations (animal breeders vs. non-breeders) in α1-globulin, β-globulin and serum total protein. Slightly higher values of total serum proteins were observed in breeders than in non-breeders. Pig breeders also presented slightly higher values of serum proteins than non-breeders, and the difference was statistically significant (Tab. 6). The same applies for the median values of albumin and total serum protein values.

In the same subjects, serum cytokines were assessed by commercially available ELISA kit. In Tables 7 and 8, the serum levels of IL-6, IL-8, IL-10, TNF-α and IFN-γ in breeders and non-breeders, and among pig and cattle breeders are shown. In comparison with control subjects, increased median levels of TNF-α, IL-8 and IL-10 were observed in animal breeders, and the difference of these parameters were statistically significant between these 2 groups while median serum levels of IL-6 and IFN-γ did not significantly change (Tab. 7).

Among the 2 breeder subgroups, a statistically significant difference in the median serum concentrations of TNF-α and

Table 5. Serum proteins in animal breeders and controls expressed in g/dl

Variable	Animal breeders (n = 64)		Non-breeders (n=32)		p-value
	Median	Min-Max	Median	Min-Max	
Albumin	4.62	3.92-5.67	4.55	4.16-5.20	0.28
α1	0.14	0.9-0.22	0.13	0.9-0.20	0.03
α2	0.71	0.53-0.87	0.68	0.54-0.86	0.06
β	0.93	0.71-1.20	0.85	0.61-1.26	0.04
γ	1.10	0.79-2.05	1.01	0.53-1.67	0.40
Total serum protein	7.48	1.39-9.03	7.30	6.60-8.03	0.04

Table 6. Serum proteins in pig breeders and cattle breeders expressed in g/dl

Variable	Pig Breeders (n = 28)		Cattle Breeders (n=36)		p-value
	Median	Min-Max	Median	Min-Max	
Albumin	4.79	3.92-5.67	4.48	3.96-5.36	<0.01
α1	0.14	0.09-0.21	0.15	0.11-0.22	0.23
α2	0.70	0.57-0.87	0.71	0.53-0.86	0.82
β	0.93	0.74-1.19	0.91	0.71-1.20	0.50
γ	1.12	0.79-1.84	0.99	0.79-2.05	0.34
Total serum protein	7.77	6.34-8.82	7.42	1.39-9.03	<0.01

Table 7. Immune serum parameters between breeders and non-breeders expressed in pg/ml (control group)

Variable	Animal breeders (n = 64)		Non breeders (n=32)		p-value
	Median	Min-Max	Median	Min-Max	
IFN-γ	10.8	7.8-41.8	10.5	8.6-16.7	0.29
TNF-α	190.1	113.8-18076.9	147.7	39.1-9858.2	<0.01
IL-10	44.4	26.3-9481.1	34.6	27-6515.1	<0.01
IL-8	41.3	27.9-168.4	31.7	14.6-55.3	<0.01
IL-6	5	3.6-142.8	4.55	3.4-7.5	0.10

IL-10 was observed, with the highest levels found in the pig breeders (Tab. 8; Fig. 1).

In Figure 1, the minimum values, lower quartile (Q1), median, upper quartile, (Q3) and the maximum values of

Table 8. Immune serum parameters between pig breeders and cattle breeders expressed in pg/ml (control group)

Variable	Pig breeders (n = 28)		Cattle breeders (n=36)		p-value
	Median	Min-Max	Median	Min-Max	
IFN-γ	11	7.6-41.8	10.35	8.3-19.9	0.06
TNF-α	215.6	113.8-18076.9	171.55	122.1-17578.3	0.04
IL-10	78	27-9481.1	38.6	26.3-8587.5	0.01
IL-8	52.1	28.5-154.2	38.5	27.9-168.4	0.15
IL-6	5.2	4.1-142.8	4.8	3.6-80.4	0.18

the measured cytokines between breeders and non-breeders are shown through box plots. Outliers and extreme values are represented with circles (°) and asterisks (*), respectively.



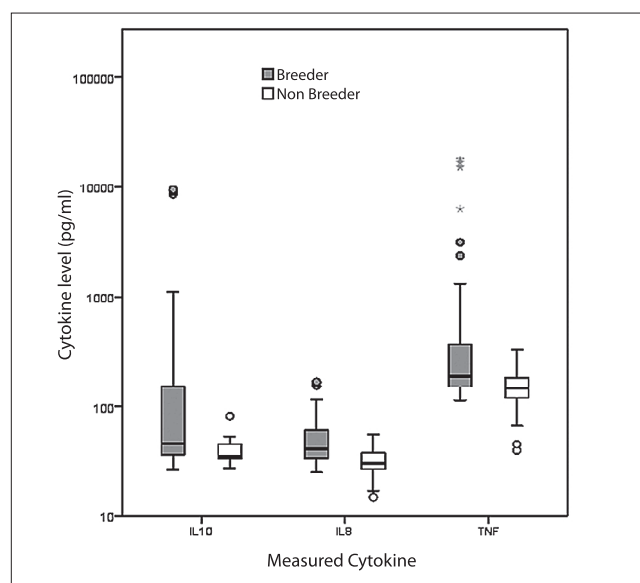


Figure 1. Serum cytokines in animal breeders and non-breeders

No significant correlation was found between serum proteins and serum cytokines. Overall, the data presented show that the serum levels of the pro-inflammatory cytokines TNF- α and IL-8, and the anti-inflammatory cytokine IL-10 were significantly increased in animal breeders.

DISCUSSION

The main finding of the presented pilot study was a statistically significant increase in the serum concentrations of TNF- α , IL-8 and IL-10 in animal breeders compared with 'non-breeders', together with a slight increase in α 1-globulin, β -globulin and total serum proteins, while no changes were observed in serum levels of IL-6 and IFN- γ or white blood cell counts. Altogether, our findings suggest that breeders showed a condition of immune system activation, and that this condition is particularly evident in pig breeders. These findings are consistent with literature data, showing that organic dusts exposure in pig breeders represents a respiratory health hazard [18, 19, 20, 21]. Several studies report an increase of the number of inflammatory cells, predominantly neutrophilic granulocytes in the deep lung, as assessed by bronchoalveolar lavage, and a release of TNF- α , IL-6 and IL-1 β in the airways of pig breeders [14, 22]. An increase of serum levels of IL-6 and TNF- α was also observed in the healthy subjects as a consequence of a few hours exposure to pig breeding farm dusts. In some studies, the increase of serum interleukins was observed within a few hours after exposure (beginning vs. end of shift) [23, 24]. Interestingly, such changes were more prominent in winter, when the windows of the breeding farms are closed, strongly suggesting the role of indoor environmental contaminants, in particular organic dusts and endotoxins [17, 25, 26].

The increase of cytokines such as TNF- α and IL-8 and airway inflammation has been reported in some studies [27, 28]. In addition, exposure to pig dusts has been shown to cause symptoms such as fever, headache and malaise, accompanied by intense airway inflammation and an influx of inflammatory cells into the upper and lower airways [14, 28, 29]. Our data indicate that the observed changes, in the

absence of any sign of health impairment, might be indicative of a risk of evolution into airway inflammation. Increased serum cytokines might be interpreted as immune changes indicative of lung inflammation that may potentially evolve into overt disease, such as ODS [11].

Several studies proved that serum protein concentrations may be altered as a result of different disease states, and the interpretation of serum protein electrophoretic patterns can be helpful in confirming the diagnosis of some diseases [30]. The results of the presented study show a statistically significant difference between animal breeders and non-breeders serum concentration of α 1, β -globulin and total serum protein. This might be due to increased exposure to biological agents in animal breeders (e.g. acute/chronic infections etc.). In this study, increase in serum proteins seems consistent with increased serum cytokines. A difference among pig and cattle breeders was observed: bigger changes were found in the pig breeders. This may be due to the phase of pig weighing, which is typically associated with a high degree of agitation and aero-solization of and exposure to air-borne dusts. Furthermore, air sampled from pig confinement buildings contains grain dusts, ammonia, fungi and bacteria, mostly Gram-positive, but also Gram-negative bacteria, typically related with ODS.

The role of airborne endotoxins in modulating these immune changes is under discussion. Another study has shown that pig farmers with less than 5 years of work had a higher prevalence of organic dust toxic syndrome than those who had been working as farmers for a longer time, suggesting the possibility of an adaptation mechanism [20]. Endotoxin might also play a role in this adaptation, since it is known that repeated exposure to these substances may result in an attenuation of the inflammatory response, referred to as 'endotoxin tolerance' [15, 31]. This may be associated with the increased level of IL-10 we observed in animal breeders. Moreover, it should be taken into account that animal feeds contain high amounts of proteins from soybean in which specific mitogens such as lectins can be present; the immune reactivity of pollens should also be taken into consideration.

CONCLUSIONS

In conclusion, occupational exposure to organic dusts induces inflammatory responses and the apparent condition of immune system activation measurable in serum levels, which might evolve into ODS. In the presented pilot study, it was found that animal breeders have a higher risk of immune system activation, and that pig breeders are a subgroup particularly at risk. More intensive investigations, especially in pig breeding facilities, involving higher numbers of workers, and collecting key environmental parameters, such as quantity and quality of the main indoor airborne contaminants, are necessary. The prognostic significance of the observed changes may be clarified through the collection of retrospective epidemiological data, as well as during the continuous health surveillance of animal breeders, with particular attention to any immune system and respiratory system changes.

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