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Publisher: Taylor & Francis

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Grana

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/sgra20>

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Available online: 05 Nov 2010

To cite this article: J. A. Amigot Lázaro, T. Díez-Ticio Ferrer, J. F. González Cabo, C. Lara Gargallo, C. Bárcena Asensio & A. A. Rodríguez Moure (2010): An aerobiological study in the rural areas of Aragon (Spain) with a high population of pigs, *Grana*, 39:5, 259-265

To link to this article: <http://dx.doi.org/10.1080/00173130052017307>

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An aerobiological study in the rural areas of Aragon (Spain) with a high population of pigs

J. A. AMIGOT LÁZARO, T. DÍEZ-TICIO FERRER, J. F. GONZÁLEZ CABO, C. LARA GARGALLO, C. BÁRCENA ASENSIO and A. A. RODRÍGUEZ MOURE

Amigot Lázaro, J. A., Díez-Ticio Ferrer, T., González Cabo, J. F., Lara Gargallo, C., Bárcena Asensio, C. & Rodríguez Moure, A. A. 2000. An aerobiological study in the rural areas of Aragon (Spain) with a high population of pigs. – Grana 39: 259–265. ISSN 0017-3134.

The development of intensive livestock farming in the swine industry has created problems associated not only with disease control but also with the disposal of excreta and other waste products. Both waste products and animals can contaminate the air of confinement swine buildings and their surroundings. The objective of this work was to determine the concentration and species composition of the bacterial and fungal microflora in the air of different villages with a high population of pigs in their surroundings. Outdoor air sampling was conducted in 24 rural urban nucleus of Aragon (Spain). In each site, three sampling points were done: one in the center of the town, another in the outskirts of the same town, and the last one in the surrounding farms. Microorganisms were isolated with a sampler Surface Air System (S.A.S.).

In the quantitative analysis, the potential risk of exposure to airborne microorganisms decreased outside and near of the farms and, of course, in other sampling points, because the airborne levels of total bacteria and fungi found in the three points are similar, slightly higher in the surrounding farms and lower inside of the buildings. The genera *Staphylococcus* and *Bacillus* among isolated Gram-positive bacteria were the most common, while among the Gram-negative genera often were *Alcaligenes* and *Acinetobacter* in most cases observed. In fungi, the most isolated genera were *Cladosporium*, *Aspergillus*, *Penicillium* and *Alternaria*, which are very important aeroallergens. In this work, were found no differences between the three sampling, which are indicative of few microbial contamination of the air from swine confinement buildings at short distances.

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(Manuscript accepted 15 December 2000)

Air contains gases, water droplets, microscopic and submicroscopic particles of pollen and dust, and microorganisms (bacteria, moulds, yeast and viruses). The microorganisms cannot grow or multiply because of the absence of nutrients, but they can be transmitted in air for long distances (Al Dagal & Fung 1990).

The development of intensive livestock farming has created problems associated not only with disease control but also with the disposal of excreta and other waste products of this industry. These waste products can contaminate the air of animal houses and their surroundings. This air is polluted with large quantities of different biohazards, including bacteria, fungi, harmful microbial products (endotoxins, enzymes, glucans) and allergens of plants and animal origin. In this way, it has resulted in increases in the potential physical, chemical and biological health hazards both to agricultural workers and to the people in the surroundings of the farms, as these agents may cause respiratory disorders (asthma, allergic aveolitis, organic dust toxic syndrome) in farm workers and in housed animals (Dutkiewicz et al. 1994)

The development of pulmonary disease, particularly allergic alveolitis (hypersensitivity pneumonitis) has been shown

to be a risk from exposure to airborne microorganisms, mainly from Gram-negative bacteria or thermophilic actinomycetes (Clark et al. 1983). Also, the abundance of fungal spores in the air has provoked lively debate regarding their potential allergenicity. Some fungi have been shown to stimulate specific antibody production. Some temporal relationship must exist between exposure to an airborne particle type and symptoms in an individual to suggest a cause-effect relationship (Burge 1986). Finally, the airborne particles of fungi also may produce cancer, rhinitis, systemic infections, headaches and mucous membrane irritants (Seltzer 1994).

The objective of this work was to determine the concentration and species composition of the bacterial and fungal microflora of air in different villages with a high population of pigs and their surroundings at three sampling points, and to compare with the results obtained inside farms by other authors.

MATERIALS AND METHODS

Outdoor air sampling was conducted in 24 rural settlement nuclei of Aragon (Spain) with a high population of pigs in their surroundings. These nuclei have a human population between 565 and 4060

residents, with a porcine population between 1350 to 24000 animals. In each sample, three points of sampling were studied: one in the center of the village, another in the outskirts of the same village in the direction of the maximum concentration of farms (approximately at 50m from the house), and the last one in the one of the satellite farms (outside, between 5 and 10m from building). The air samples were obtained under good meteorological conditions (lack of wind and rain) and a height of 145cm.

Microorganisms were isolated with a sampler Surface Air System (S.A.S.; International PBI, Milano, Italy). This sampler has a nominal flow rate of 180l/min and is powered by a portable 12V battery. In this work a sampling time of 60 seconds was selected, thus 180 litres of air has been taken in this time for each plate, it proved best period for the individual identification of colonies in the plates exposed in outdoor air. The sampling head (65×15mm) has 219 holes (Ø1mm). To collect particles, Replicate Organism Detection and Counting (RODAC[®], Becton Dickinson, Cockeysville, USA) plates filled with approximately 13ml of agar were used under the sampling head.

Bacteria

For bacteria isolation were taken the following media: Plate Count agar (DIFCO Laboratories, Detroit, USA) for total aerobic bacterial counts, for duplicate, Broth Azide Dextrose (DIFCO) with 13% of Agar-Agar (DIFCO) for isolation of *Enterococcus*, Chapman Agar n° 110 (DIFCO) for isolation of *Staphylococcus*, Agar G.S.P. base (MERCK) with 100.000 i.u. of Penicillin G and 0.4ml of suspension 2.5% Pimafucin for isolation of *Pseudomonas* and *Aeromonas* and finally, MacConkey agar (DIFCO) for isolation of Enterobacteriaceae.

In order to isolate as wide a spectrum of bacteria as possible, Plate Count agar plates were incubated after exposure: one at 37°C for 48 hours and other at 22°C for 72 hours. Plates of Broth Azide with Agar-Agar, Chapman and MacConkey agars were incubated at 37°C for 48 hours. Finally, Agar G.S.P. was incubated at 30°C for 72 hours.

Total bacteria were counted on Plate Count and different colonies on this media also were identified to species level. Colonies were then counted and identified and the data were reported as colony forming units (CFU) m⁻³ of air.

All colonies growing on MacConkey, Chapman, G.S.P. agars and Azide with agar were reinoculated on fresh media (Brain Heart Infusion agar, DIFCO) for further identification.

Identification of microorganisms was done using API STAPH (for *Staphylococcus* and *Micrococcus*), API 20E (for Enterobacteriaceae), API 20NE (for Non enteric Gram-negative rods), API STREP (for *Streptococcus*) (BioMérieux, France), and several tests from Cowan and Steel's Manual (by Barrow & Feltham 1995).

Fungi

For fungal isolation were taken the following medium: Sabouraud Chloramphenicol-agar (PASTEUR). The plates were incubated at 28–30°C for 5–7 days. Colonies were then counted and identified and the data were reported as colony forming units (CFU) m⁻³ of air.

All colonies growing were then reinoculated on fresh medium of the same type for further investigation using identification keys (Ainsworth et al. 1973, Von Arx 1981, Ellis 1971, 1976, McGinnis 1980). In other cases, the fungal genera were identified using the microculture technique (Gonzalez 1985) and later identification with keys. The colonies were identified to genus level and the microorganisms, included in the genus *Aspergillus*, in same cases, to species level.

The identification of yeast was done using the API AUX System (BioMérieux, France).

RESULTS

Quantitative analysis

The descriptive analysis of airborne concentrations of microorganisms detected at three sampling points are presented in Table I.

The airborne concentration of total bacteria at 22°C was higher than at 37°C and some airborne bacterial levels were moderately higher in the satellite farms. Also, the airborne concentration of total fungi in the satellite farms was higher than in the center of the village and the outskirts.

Qualitative analysis

A high diversity of genera and species was found in the bacteria grown on the several media used. The numbers of genera found in the three points are shown in the Table II and the different species identified within each genus are shown in the Table III.

Identification of the colonies isolated revealed a great diversity of bacteria present in the air of the different sampling points. The genera *Staphylococcus* and *Bacillus* were the most numerous among isolated Gram-positive bacteria, while the Gram-negative genera most frequently identified were *Alcaligenes* and *Acinetobacter*.

The numbers of fungal genera found at the three sampling points, both filamentous fungi and yeast, are shown in the Table IV. The specific composition of selected fungi (*Aspergillus* and yeast) are shown in the Table V.

The genera *Aspergillus*, *Penicillium* and *Alternaria* were the most numerous among the filamentous fungi while the yeast genera *Candida* and *Rhodotorula* were most frequently identified.

DISCUSSION

Bacteria

Airborne concentrations of bacteria found in this study were comparable to those previously reported by Bovallius et al. (1978a) with 7.4×10¹ CFU/m³ of air in cultivated land and 3.9×10¹ CFU/m³ in the coast. Bovallius et al. in a later work (1978b) found 3.4×10³ and 5.6×10² CFU/m³ respectively in the same cases. Also in settlement nuclei he determined 7.6×10² and 8.5×10² CFU/m³ respectively in a park and the street of the village.

Higher amounts were reported by Rahnoken (1992) with 7×10⁴ CFU/m³ in the surrounding wastewater treatment plants. Also Laitinen et al. (1992) in these plants collected 10² to 10⁵ CFU/m³. Less amounts were by found Jones & Cookson (1983) in depuration plants with 7.5×10¹ CFU/m³ of mesophilic bacteria.

In this case, the swine confinements buildings could be the cause of the spreading of bacteria externally, because inside these barns the concentration of mesophilic bacteria in the air is very high, despite that the majority of the farms in Aragon (Spain) have natural ventilating systems. These microorganisms derived from feed, desquamated epithelial skin cells, dried faeces and dried urine.

It has been reported that concentrations of mesophilic bacteria in pig confinement houses ranged from 2×10⁵ to

Table I. Descriptive analysis of bacterial total counts (at 22°C and 37°C) and fungal total counts at three sampling points (CFU/m³).

	Center			Outskirts			Farms		
	B-22	B-37	F	B-22	B-37	F	B-22	B-37	F
Descriptive analysis									
n	24	24	24	24	24	24	21	21	21
Arithmetical mean	774.1	266.3	239.7	820.2	391.0	280.0	991.0	443.4	296.4
Standard deviation	475.5	223.8	185.9	451.0	429.5	268.6	528.4	469.9	226.1
Standard error	97.1	45.7	37.9	92.0	87.7	54.8	115.3	102.5	49.3
Variation coefficient	61.4	84.1	77.6	55.0	109.9	95.9	53.3	106.0	76.3
Minimum	137	44	17	11	93	11	209	33	6
Maximum	1650	935	710	1650	1650	1100	1650	1650	869

B-22 = bacterial total counts at 22°C.

B-37 = bacterial total counts at 37°C.

F = fungal total counts.

Table II. Number of bacterial strains isolates in the 24 samples.

Bacterial genera	Center	Outskirts	Farms	TOTAL	%
<i>Acinetobacter</i>	11	14	11	36	5.4
<i>Actinomyces</i>	9	7	2	18	2.7
<i>Aerococcus</i>	7	5	7	19	2.9
<i>Alcaligenes</i>	12	13	11	36	5.4
<i>Bacillus</i>	64	57	54	175	26.2
<i>Chromobacterium</i>	4	5	2	11	1.7
<i>Citrobacter</i>	1	1	0	2	0.3
<i>Comamonas</i>	2	0	1	3	0.4
<i>Corynebacterium</i>	9	4	3	16	2.4
<i>Edwardsiella</i>	1	0	0	1	0.1
<i>Enterobacter</i>	5	5	1	11	1.7
<i>Enterococcus</i>	11	5	8	24	3.6
<i>Flavobacterium</i>	1	3	6	10	1.5
<i>Kurthia</i>	2	2	1	5	0.7
<i>Micrococcus</i>	22	23	13	58	8.7
<i>Pseudomonas</i>	4	14	4	22	3.3
<i>Serratia</i>	1	2	0	3	0.4
<i>Staphylococcus</i>	89	74	52	215	32.2
<i>Xanthomonas</i>	0	2	1	3	0.4
TOTAL	255	236	177	668	100

3.6×10⁵ CFU/m³ in Sweden (Clark et al. 1983), from 1.5×10⁵ to 5.4×10⁵ CFU/m³ in Canada (Cormier et al. 1990), from 2×10⁵ to 8×10⁶ CFU/m³ in United Kingdom (Crook et al. 1991), from 7.3×10⁴ to 9.6×10⁴ CFU/m³ in USA (Thorne et al. 1992) and from 5.6×10⁵ to 1.2×10⁶ CFU/m³ in farms operating in Poland (Dutkiewicz et al. 1994).

The farm workers and housed animals can be exposed to large quantities of airborne microorganisms and their products, which create a potential risks of respiratory disease due to their immunomodulatory action inside the farms (Dutkiewicz et al. 1994).

In this study, evidently, the concentration of mesophilic bacteria near of the farms is lower that inside of the buildings with 9.9×10² CFU/m³ at 22°C and 4.4×10² CFU/m³ at 37°C, but are higher that in the center of village with 7.7×10² CFU/m³ at 22°C and 2.6×10² CFU/m³ at 37°C and in the outskirts of the same village (in the direction of the maximum concentration of farms) with 8.2×10² CFU/m³

at 22°C and 3.9×10² CFU/m³. However, the differences between these three sampling points are minimal.

In all sampling points the percentage of Gram-positive bacteria was between 75% and 83%; and Gram-negative bacteria between 17% and 25%. The average percentage was 80% and 20% respectively. Inside farms, this percentage can vary, from 30% (Clark et al. 1983) to 53% (Cormier et al. 1990) of Gram-negative bacteria.

Airborne mesophilic bacteria investigated in this study were predominantly Gram-positive, mostly of the genera *Staphylococcus* and *Bacillus*. Gram-positive cocci, mostly coagulase-negative strains of *Staphylococcus*, were the most significant. Inside farms, these genera are of frequent occurrence too. According to Cormier et al. (1990) *Staphylococcus*, *Bacillus* and *Aerococcus* are the most frequently isolated genera. Crook et al. (1991) showed that in more than 75% of all samples are Gram-positive cocci the most frequent group.

In the air of urban environments appear also Gram-positive bacteria, with 41% of *Micrococcus*, 11% of *Staphylococcus* and 8% each of *Aerococcus* and *Bacillus*, as reported by Mancinelli & Shulls (1978). The majority of bacteria Gram-positive isolated were those associated with mammalian skin (Sneath et al. 1986).

In the group of Gram-negative bacteria the most frequent genera were *Alcaligenes*, *Acinetobacter* and *Pseudomonas*. The potential health hazards from microbial aerosols of this group is the possibility to produce endotoxins with potential risk for workers exposed to its inhalation (Burrell & Ye 1990). In the animal houses, the concentration of endotoxins is very high (Clark et al. 1983) but in the out-door air the endotoxins are not frequent.

The microorganism *Escherichia coli*, which is indicatory bacterium of faecal contamination, was not isolated in this work. Noteworthy *Enterobacter agglomerans* has been isolated in 11 samples. That corresponds to results obtained by Cronholm (1980) with this microorganism, mainly isolated in the proximity of activated sludge plants. It may indicate that *Enterobacter* could be better indicator of the atmospheric bacterial contamination's sources (in comparison with *Escherichia coli*).

Fungi

Quantitatively, the total counts of airborne fungal spores found in this study were comparable to proceeding studies,

Table III. Percentages of species within each genus of bacterial isolates.

Genera	Species	%
<i>Acinetobacter</i>	<i>A. calcoaceticus</i>	72
	<i>A. xylosoxidans</i>	3
	<i>A. paradoxus</i>	36
<i>Bacillus</i>	<i>A. faecalis</i>	61
	<i>B. globisporus</i>	1
	<i>B. macquariensis</i>	1
	<i>B. fastidiosus</i>	1
	<i>B. panthotenticus</i>	2
	<i>B. alvei</i>	2
	<i>B. lentus</i>	2
	<i>B. polymyxa</i>	2
	<i>B. schlegelli</i>	3
	<i>B. azotoformans</i>	3
	<i>B. cereus</i>	3
	<i>B. laterosporus</i>	3
	<i>B. megaterium</i>	3
	<i>B. brevis</i>	4
	<i>B. pumilus</i>	5
	<i>B. licheniformis</i>	6
	<i>B. coagulans</i>	6
	<i>B. thuringiensis</i>	7
	<i>B. sphaericus</i>	10
	<i>B. circulans</i>	15
	<i>B. mycoides</i>	21
	<i>C. violaceum</i>	36
<i>Chromobacterium</i>	<i>C. fluviatile</i>	64
<i>Enterobacter</i>	<i>E. gergoviae</i>	9
	<i>E. aerogenes</i>	9
<i>Enterococcus</i>	<i>E. agglomerans</i>	82
	<i>E. gallinarum</i>	12
	<i>E. faecium</i>	13
	<i>E. faecalis</i>	75
<i>Micrococcus</i>	<i>M. nishinomiyaensis</i>	2
	<i>M. lylae</i>	5
	<i>M. agilis</i>	5
	<i>M. sedentarius</i>	10
	<i>M. kristinae</i>	14
	<i>M. luteus</i>	19
	<i>M. roseus</i>	21
	<i>M. varians</i>	24
	<i>P. vesicularis</i>	5
	<i>P. paucimobilis</i>	9
<i>Pseudomonas</i>	<i>P. fluorescens</i>	9
	<i>P. stutzeri</i>	27
	<i>P. cepacia</i>	50
	<i>S. rubidaea</i>	3
	<i>S. liquefaciens</i>	33
<i>Serratia</i>	<i>S. fonticola</i>	34
	<i>S. epidermidis</i>	1
<i>Staphylococcus</i>	<i>S. carnosus</i>	1
	<i>S. auricularis</i>	2
	<i>S. sciuri</i>	2
	<i>S. saprophyticus</i>	2
	<i>S. hominis</i>	4
	<i>S. chromogenes</i>	5
	<i>S. cohnii cohnii</i>	5
	<i>S. gallinarum</i>	6
	<i>S. hyicus</i>	6
	<i>S. cohnii urealyticus</i>	6
	<i>S. capitis</i>	7
	<i>S. haemolyticus</i>	7
	<i>S. lentus</i>	9
	<i>S. simulans</i>	14
	<i>S. xylosus</i>	23

Table IV. Number of fungal strains isolates in the 24 samples.

MOLDS	Center	Outskirts	Farms	TOTAL	%
<i>Acronium</i>	6	1	4	11	4.1
<i>Alternaria</i>	16	15	13	44	16.6
<i>Aspergillus</i>	29	16	17	62	23.3
<i>Cladosporium</i>	11	12	8	31	11.7
<i>Dreschlera</i>	1	2	1	4	1.5
<i>Fusarium</i>	0	0	2	2	0.7
<i>Mycelia sterilia</i>	7	5	6	18	6.8
<i>Dematiaceous.</i>	3	1	1	5	1.9
<i>myc. ster.</i>					
<i>Monilia</i>	0	1	1	2	0.7
<i>Mucor</i>	2	3	3	8	3.0
<i>Paecilomyces</i>	0	1	1	2	0.7
<i>Penicillium</i>	18	16	15	49	18.4
<i>Rhizopus</i>	2	3	2	7	2.6
<i>Scopulariopsis</i>	3	1	1	5	1.
<i>Sincephalastrum</i>	0	1	0	1	0.4
<i>Stemphylium</i>	1	0	0	1	0.4
<i>Trichoderma</i>	1	0	0	1	0.4
<i>Ulocladium</i>	4	3	5	12	4.5
<i>Verticillium</i>	0	0	1	1	0.4
TOTAL	104	81	81	266	100
YEASTS					
<i>Candida</i>	1	4	2	7	43.75
<i>Cryptococcus</i>	0	1	1	2	12.5
<i>Rhodotorula</i>	2	1	1	4	25
<i>Torulopsis</i>	2	1	0	3	18.75
TOTAL	5	7	4	16	100

Table V. Specific composition of selected fungi.

Fungi	Species	%
<i>Aspergillus</i>	<i>A. flavus</i>	8.1
	<i>A. ochraceus</i>	17.8
	<i>A. niger</i>	19.4
	<i>A. fumigatus</i>	24.1
	<i>A. spp.</i>	30.6
Yeasts	<i>Candida lusitanae</i>	6.2
	<i>Cryptococcus albidus diffuens</i>	6.2
	<i>Cryptococcus albidus albidus</i>	6.2
	<i>Candida humicola</i>	12.6
	<i>Torulopsis candida</i>	18.8
	<i>Candida guilliermondii</i>	25.0
	<i>Rhodotorula rubra</i>	25.0

e.g., Beaumont et al. (1985a) who reported 2.6×10^2 CFU/m³ in land, and Rantio-Lehtimäki (1985), who found 2.5×10^2 CFU/m³ in the comparable site.

Lower quantities were reported by Gonzalez-Minero et al. (1992, 1993) in Cadiz and Huelva (Spain) with 1.5×10^2 and 6×10^1 CFU/m³ of the air respectively.

In similar studies, larger quantities were reported by Caretta et al. (1983) that isolate 6×10^2 CFU/m³ air in Pavia (Italy). Eversmeyer & Kramer (1975) found 3×10^4 CFU/m³ within the wheat land while Larsen (1981) describe results from 2×10^1 to 1×10^3 CFU/m³ in Copenhagen (Denmark). Kramer & Eversmeyer (1984) found 1.5×10^3 to 2.6×10^4 CFU/m³ within (or just outside) the city of Manhattan (Kansas, USA). Ebner et al. (1989) reported until 3.5×10^3 CFU/m³ in the Inn Valley (Austria), Ebner et al. (1992) up

to 2×10^3 CFU/m³ in the neighborhood of university in Innsbruck (Austria).

In an review article reported Pasanen (1992) mean values of viable spore counts in residential environments in Europe and America from 4×10^1 to 2.6×10^3 CFU/m³ in eighteen studies. These results, in several sites, are very variable. The composition and concentration of airborne fungal spores are, therefore, largely determined by geographical location, meteorological factors, vegetation, and human activities, as well as by a wide range of interrelated environmental and biological factors (Li & Kendrick 1994). One of these factors could be the swine confinement buildings as focus of spread of fungal spores to exterior.

In waste treatment plants, with similar characteristics to farms, the counts of mesophilic fungi were variable too, in waste sorting plants they were between 2.3×10^3 and 1.1×10^4 CFU/m³, and in waste incineration plant from 4.7×10^2 to 3.2×10^4 CFU/m³ (Rahkonen 1992). In the air of sewage sludge composting site total counts of thermotolerant fungi were $2\text{--}4 \times 10^3$ CFU/m³ (Kothary et al. 1984), having been higher compared to with the total viable fungi in surroundings of farms of pigs in this study. Nevertheless, in the study of Kothary et al. (1984) the concentration of fungi in air decreased with distance, while in our study, the levels are similar in the three sampling points.

Inside the farms, Clark et al. (1983) found in swine confinement buildings 3×10^2 CFU/m³ as a mean. Cormier et al. (1990) reported in farrowing units of piggeries from 10^1 to 10^2 CFU/m³ of molds and 5×10^1 CFU/m³ of yeasts. In fattening units the results were from 4×10^1 to 1.5×10^2 CFU/m³ for moulds and 4×10^1 CFU/m³ for yeasts. Donham et al. (1986) reported, in USA and Sweden, higher level of molds, namely 1.9×10^3 and 3.0×10^4 CFU/m³. Crook et al. (1991) found that the concentration of airborne fungi in pig confinement houses, ranged from 2×10^3 to 10^5 CFU/m³. Thorne et al. (1992) comparing this three methods, found the total counts of fungi from 1.9×10^3 to 5.8×10^3 CFU/m³ in barns housing swine. Finally, Dutkiewicz et al. (1994) reported between 1.3×10^3 and 3.1×10^4 CFU/m³ of fungal spores in piggeries.

In this study, the concentration of fungal spores near of the satellite farms was 2.9×10^2 CFU/m³, very low compared with the fungal counts inside buildings, but is lightly higher than in the center of village with 2.4×10^2 CFU/m³ and the outskirts of the same village (in the direction of the maximum concentration of farms) with 2.8×10^2 CFU/m³. Nevertheless, the differences in the three sampling points are minimal and moreover, the correlation coefficients in fungal counts between each sampling point were $r=0.84$ (between center and satellite farms), $r=0.86$ (center and outskirts) and $r=0.73$ (satellitic farms and outskirts).

The concentrations of fungal spores have not significant differences in the three sampling points, and are lower than inside the farms and similar to other studies. The potential risk of respiratory disease decrease outside the farms.

Qualitatively, with only few exceptions the same three genera were found: *Aspergillus* with 62 strains, *Penicillium* with 49 strains, and *Alternaria* with 44, as most commonly at all sites. These three genera are important aeroallergens,

outdoor and indoor both, especially *Alternaria* (Horner et al. 1995).

In alpine environments, Ebner et al. (1992) found mainly *Cladosporium*, yeast, mycelia sterilia, *Epicoccum*, *Aureobasidium*, *Penicillium*, *Alternaria*, *Aspergillus*, *Botrytis* and *Phoma*, occurring commonly in the same order as presented here.

In Italy, country with similar to Spain agro-ecological characteristics, Caretta et al. (1983) reported the following genera: *Cladosporium*, *Aureobasidium* and *Alternaria* in Pavia; D'Amato et al. (1984) reported the genus *Cladosporium* as the most isolate in Naples; Fillipello Marchisio et al. (1992) isolated *Penicillium*, *Aspergillus* and *Cladosporium* in Turin. Finally, Bagni et al. (1976) reported the genera *Penicillium*, *Aspergillus*, *Chaetomium* and *Alternaria* mainly at Monte Cimone. Similarly in Greece (Athens), Bartzokas (1975) isolated mainly *Alternaria*, *Aspergillus*, *Chrysosporium*, *Mucor*, *Penicillium* and yeast. In Israel, Barkai-Golan et al. (1977) found that the main components of the airspora belong to the three genera: *Cladosporium*, *Alternaria* and *Penicillium*. These results show that the main part of air spores in Mediterranean countries, are those of *Cladosporium*, *Aspergillus*, *Penicillium* and *Alternaria*, confirm *pro parte* our results.

Inside piggeries, the incidence of these genera is strongly frequent. Cormier et al. (1990) reported *Scopulariopsis*, *Aspergillus*, *Penicillium*, and *Candida* as most frequently isolated. Crook et al. (1991) showed that the most common isolated fungi were nonpigmented yeasts, *Scopulariopsis* and *Cladosporium*. According to Dutkiewicz et al. (1994), the fungal flora of the air showed a marked diversity, so, yeasts predominated in the fattening piggeries while in farrowing houses the most common genera were: yeasts, *Penicillium*, *Aspergillus* and *Eurotium*.

The most isolated genera in this work coincide with the other findings in a rural and urban environments (*Cladosporium*, *Aspergillus*, *Penicillium* and *Alternaria*). These genera are important aeroallergens, but we found the same genera both inside barns housing swine and the outside farms.

The species of the genus *Aspergillus* very important as aeroallergens, e.g. *Aspergillus fumigatus* and *Aspergillus niger* are isolated most frequently, even in the rural and urban environments without such farms (Pasanen 1992, Filipello Marchisio et al. 1992). The same can we observe with the yeasts; that are mainly pathogenic for immunodepressed people and animals (Rippon 1990).

CONCLUSIONS

Bacteria

In conclusion, the airborne levels of total bacteria found in the three sampling points were similar and lower than inside farms, consequently the potential risk of exposure to these microorganisms decreased outside piggeries. The Gram-positive bacteria (mainly associated with mammalian skin) were most frequently isolated forming 80% of the total count.

In this work, no differences (quantitative) were found in the three sampling points, which is indicative of little bacterial contamination of air from swine confinement buildings at

short and medium distances, which in concordance with other authors.

Fungi

In this study, with respect to quantitative analysis of fungi, there were not differences between three sampling points, this is indicative of a little fungal contamination of air from swine confinement buildings at short and medium distances. Unlike bacteria, the genera of moulds (*Cladosporium*, *Aspergillus*, *Penicillium* and *Alternaria*) and yeast (*Candida* mainly) from air were similar both inside piggeries and the atmosphere of Mediterranean countries. Thus, the fungal contamination from swine confinement buildings have not environmental impact due to low fungal dispersion around the farms and is not important as aeroallergens in their surroundings.

ACKNOWLEDGEMENTS

This work was supported in part by the Diputacion General de Aragon (Spain) with a scholarship of J. A. Amigot (B-CM/91).

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