

A METHOD TO EVALUATE THE MICROBIAL AIR COMPOSITION IN RABBIT FARM

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ABSTRACT

The microbiological monitoring of air and surfaces has the purpose to evaluate the hygienic conditions of environment to be sure that the disinfecting operations are correctly performed or to decide what kind of operation would be better to assure a good environmental quality. Since environmental quality, particularly of air, is tightly correlated with animal welfare, it is very important to find methods for the evaluation of the air in animal farms. The last EU directives and EFSA opinions are quite restrictive in relation to the management and housing systems. An important topic is represented by the air pollution in relation to the microbial charge; above all it is interesting to evaluate the presence of bacteria, fungi and dermatophytes (zoonotic agents) that quickly spread from animal to animal and to human workers. In fact rabbits are particularly sensitive to environmental conditions and to stress due to changes in some parameters (e.g., temperature, relative humidity, NH₃ concentration, etc.) that can increase their susceptibility to potential pathogens.

In our experience, we have used an easy method and a working protocol in order to check and control the air contamination in an intensive rabbit farm, located in Lombardia region, analyzing the air samples inside the shed and inside the cages and nests during a period of three years (2004-2007). This method is based upon the SAS[®] System (PBI International, Italy), an impaction method by which the air is collected directly on the Petri plates filled with the chosen cultural medium. In our protocol we have decided to evaluate the total microbial charge and the fungal one. So we used as cultural media Tryptic Soy Agar (Oxoid, Italy) for bacterial growth, Sabouraud Dextrose Agar (Oxoid, Italy) for environmental fungi (such as *Aspergillus* spp., *Penicillium* spp., *Alternaria*, spp.) and Dermasel Agar (Oxoid, Italy) for dermatophytes (*Microsporum* and *Trichophyton*).

After many trials we have observed that this method, compared with the “opened plates” method, allows to obtain repeatable results in a brief time and to perform the tests with low cost. The results allow to evaluate the environmental status of rabbitries, to know the welfare of reared rabbits and to decide the hygienic operation to perform in the breeding farms.

Key words: Environmental quality, Rabbit rearing, Microbiological charge, Air sampler.

INTRODUCTION

The environmental quality is much important in the animal intensive farms because there is a tight correlation between animal welfare and healthy status and microbial concentration (contamination). In fact, in the air there are a lot of microorganisms (bacteria, viruses, fungi, parasites) that can spread easily and quickly among animals and from animals to human. Changes in some environmental parameters such as temperature (>25°C), relative humidity (>70%), NH₃ and CO₂ concentration could improve the presence of various microorganisms and stress animals (Rosell, 1986; Zaror and Casas, 1988; Pitt, 1994; Vangeel *et al.*, 2000). Moreover, the microbial charge could be high because animal overcrowding, poor hygiene conditions (Martino *et al.*, 1998; Jacchia and Martino, 2000; Luzi *et al.*, 2005; Martino *et al.*, 2007) or low healthy status of animals. Rabbits are animals often bred intensively and are particularly subordinate to some infectious diseases such as gastro-enteric, cutaneous and respiratory pathologies (Van Cutsen and Rochette, 1991; Farina and Scatozza, 1998).

The last European directives underline the importance of air pollution in relation to the microbial contamination; so it's important to evaluate the presence of bacteria, fungi, and soon.

We have developed a working protocol in order to evaluate the air contamination in different kinds of rabbitries using an easy instrument for sampling, the SAS[®] System (PBI International, Italy) and the Surfair Plate (PBI International, Italy) filled with different types of media (Mehta *et al.*, 1996; Jericho *et al.*, 2000). This method was compared with the traditional ones that used “opened plates”.

MATERIALS AND METHODS

Farm

The trials have been performed in a meat rabbit farm located in the North-West of Italy. Rabbits (New Zealand hybrids) were housed in two different rooms of the same building, with forced ventilation, one for does and one for fatteners.

Air sampling

SAS[®] System

The SAS[®] System (PBI International, Italy) is an impaction method that allows to enumerate the number of microorganisms directly in elevated convex plates (Surfair Plates, PBI International, Italy) filled with cultural media (Figure 1).



Figure 1: the SAS[®] apparatus (International PBI, Italy)

This instrument has two main components: the sampling head and the control unit/pump assembly that are integrate. A known volume of air (in adherence to manufacturer's flow rates) is thrown onto a Surfair plate; then the sample is incubated depending on the microorganisms we want to isolate. The results are expressed as CFU (Colony Forming Unit)/m³.

Opened plates

The “opened plates” method uses Petri dishes, filled with the same three cultural media as above, opened in the environment of sheds for 10 minutes and then incubated at the same temperature and the same period used for the plates obtained with the SAS[®] System.

Cultural media

Our protocol was performed using three kinds of cultural media: Tryptic Soy Agar (Oxoid, Italy), a typical medium for bacterial evaluation; Sabouraud Dextrose Agar (Oxoid, Italy), for environmental

fungi (such as *Aspergillus* spp., *Penicillium* spp., *Alternaria*, spp.) and Dermasel Agar (Oxoid, Italy), a specific medium for isolation of dermatophytes (*Microsporum* spp. and *Trichophyton* spp.). After the sampling directly with the SAS[®] System, the plates were incubated at different temperatures and time depending on the cultural behaviour of microorganisms. For bacteria we used 37°C for 24-48 hours, for environmental fungi 25°C for 72 hours and for dermatophytes 25°C for 5-7 days (2 weeks to confirm a negative result) (Quinn *et al.*, 1994; Poli *et al.*, 2005).

Identification of bacteria and fungi

The identification of the isolated bacterial strains was performed by determining the macroscopic characteristics of colonies on the Tryptic Soy Agar, by performing the Gram stain (Oxoid, Italy) (Quinn *et al.*, 1994; Poli *et al.*, 2005) and by using selective and differential media [Mac Conkey Agar (Oxoid, Italy), Mannitol Salt Agar (Oxoid, Italy)].

The fungal identification was performed by evaluating the macroscopic and microscopic characteristics of the colonies (pigmentation; dry or cottony aspect; *macroconidia* and *microconidia*) (Quinn *et al.*, 1994; Poli *et al.*, 2005).

RESULTS AND DISCUSSION

During the first trials developed in the rabbit farm, the bacterial charge we have observed was always above 50 CFU/m³ of aspired air and about the same value was obtained with opened plates; *Micrococcus luteus* and *Staphylococcus* spp. were the most isolated microorganisms in both kind of samples. For fungi, 100% of plates were positive for environmental fungi and yeasts such as *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp. and *Rhodotorula rubra* but their number remained under 50 CFU/m³. Opened plates have shown a similar qualitative result while it was more difficult to count fungal colonies due to the presence of rabbit coat on plates. The percentage of dermatophytes isolated was between 50 and 70 and the only species identified was *Trichophyton mentagrophytes* as in opened plates (Table 1).

Table 1: Airborne microbial contamination: results of the two trials

First trial (2004-2005)					
Microorganisms	SAS System			Opened plates	
	Positive samples (%)	CFU/m ³	Identification	Positive samples (%)	Identification
Bacteria	100	>50	<i>M. luteus</i> <i>Staphylococcus</i> spp.	100	<i>M. luteus</i> <i>Staphylococcus</i> spp.
Environmental fungi/yeast	100	<50	<i>Aspergillus</i> spp. <i>Penicillium</i> spp. <i>Alternaria</i> spp. <i>Rhodotorula rubra</i>	100	<i>Aspergillus</i> spp. <i>Penicillium</i> spp. <i>Alternaria</i> spp.
Dermatophytes	50-70	<50	<i>Trichophyton mentagrophytes</i>	100	<i>Trichophyton mentagrophytes</i>
Second trial (2006-2007)					
Microorganisms	SAS System			Opened plates	
	Positive samples (%)	CFU/m ³	Identification	Positive samples (%)	Identification
Bacteria	100	50-100	<i>Bacillus</i> spp.	100	<i>Bacillus</i> spp.
Environmental fungi	47.21	<50	<i>Aspergillus niger</i> <i>Alternaria</i> spp.	100	<i>Aspergillus niger</i> <i>Alternaria</i> spp.
Dermatophytes	41.67	<50	<i>Microsporum gypseum</i>	100	<i>Microsporum gypseum</i>

During the second trials, the microbiological results showed a total bacterial charge between 50 and 100 CFU/m³ air flow, using the SAS[®] System. On the contrary, the total fungal charge (environmental fungi and dermatophytes), keeps below 50 CFU/m³ (Table 1). These values could be considered low (50-100 CFU/m³) and very low (<50 CFU/m³) with regards to the environmental risk according to the

parameters supplied by the SAS System's producer (AA.VV., 1991). The isolated microorganisms were *Bacillus* (100%), *Alternaria* (11,09%), *Aspergillus niger* (36.12%) and *Microsporum gypseum* (41.67%). The same microorganisms were qualitatively identified with opened plates (Table 1).

So, the two methods we have compared for the evaluation of the microbial air quality allow us to obtain superimposed results but the data of SAS[®] System are both qualitative and quantitative while opened plates permit only a qualitative result. Moreover, the set of opened plates could stress animals that are timid and curious and it is impossible to use them in nests; on the contrary the SAS[®] System is portable and noiseless and it is optimal for sampling air in the nests.

CONCLUSIONS

Our aim was to evaluate a method for the control of the microbial air composition in rabbit farm, compared with the classic method of "opened plates" that we have used many times in the past. We have used the SAS[®] System (PBI International, Italy), a simple and cheap method. The results, which we have collected during a period of three years, have proven that this impact method is good, exact and reproducible; furthermore it does not stress animals when it is used with rabbits in cages too.

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