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**Concentration of airborne *Staphylococcus aureus* (MRSA and MSSA), total bacteria and endotoxins in pig farms.**

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**ABSTRACT**

Pigs are very often colonized with *Staphylococcus aureus* and transmission of such pig-associated *S. aureus* to humans can cause serious medical, hygiene and economic problems. The transmission route of zoonotic pathogens colonizing farm animals to humans is not well established and bioaerosols could play an important role. The aim of this study was to assess the potential occupational risk of working with *S. aureus*-colonized pigs in Switzerland. We estimated the airborne contamination by *S. aureus* in 37 pig farms (20 nursery and 17 fattening units; 25 in summer, 12 in winter). Quantification of total airborne bacteria DNA, airborne *Staphylococcus sp* DNA, fungi, and airborne endotoxins were also performed. In this experiment, the presence of cultivable airborne methicillin-resistant *S. aureus* (MRSA) CC398 in a pig farm in Switzerland was reported for the first time. Airborne methicillin-sensitive *S. aureus* (MSSA) was found in approximately 30% of farms. The average airborne concentration of DNA copy number of total bacteria and *Staphylococcus* species measured by qPCR was very high, respectively reaching values of  $75 (\pm 28) \times 10^7$  copy number/m<sup>3</sup> for total bacteria and  $35 (\pm 9.8) \times 10^5$ /m<sup>3</sup> for *Staphylococcus sp* in summer and  $96 (\pm 19) \times 10^8$  copy number/m<sup>3</sup> for total bacteria and  $40 (\pm 12) \times 10^6$  copy numbers/m<sup>3</sup> for *Staphylococcus sp*. in winter. Total mean airborne concentrations of endotoxins (1298 UE/ m<sup>3</sup>) and fungi (5707 CFU/m<sup>3</sup>) exceeded the Swiss recommended values and were higher in winter than in summer. In conclusion, Swiss pig farmers will have to tackle a new emerging occupational risk which could also have a strong impact on public health. The need to inform pig farmers about biological occupational risks is therefore crucial.

## INTRODUCTION

Farm animals are colonized by a co-evolved specific bacterial community. In particular, the nostril (anterior nares) is an important niche for bacterial colonization by both opportunistic pathogens and commensals. A study performed in France (Armand-Lefevre *et al.*, 2005) was the first to show that a particular clonal complex of Methicillin Resistant *Staphylococcus aureus* (MRSA), namely CC398, was able to colonize healthy pig farmers and pigs. A subsequent worrying report indicated that 40% of pigs from Holland carried MRSA CC398 in their nostrils (de Neeling *et al.*, 2007, van Duijkeren *et al.*, 2008). This observation has been confirmed by a number of studies in other European countries including Belgium (Denis *et al.*, 2009), Denmark (Guardabassi *et al.*, 2007) and Germany (Witte *et al.*, 2007) but also Canada (Khanna *et al.*, 2008), the USA (Smith *et al.*, 2009) and Singapore (Sergio *et al.*, 2007). Several studies report that MRSA CC398 was involved in serious clinical infections affecting patients in close contact with pigs (Witte *et al.*, 2007; Declercq *et al.*, 2008; van Loo *et al.*, 2007; Lewis *et al.*, 2008; Van Hoecke *et al.*, 2009; Lozano *et al.*, 2011). Even if MRSA CC398 represents a new occupational risk that needs to be prevented, no studies have investigated the problem from an occupational health point of view. Most of the studies associated with pig MRSA have focused their attention only on nasal carriage of pigs, pig farmers and veterinarians without investigating routes of transmission or factors, which can influence the prevalence in animal farms and without investigating the airborne prevalence of other clonal complexes of *S. aureus*. However, apart from MRSA, pigs are also colonised with a specific *S. aureus* CC398 genotype not resistant to methicillin called Methicillin Sensitive *Staphylococcus aureus* (MSSA). A recent study of our group has demonstrated that although prevalence of nasal carriage of MRSA CC398 in pigs and pig farmers was very low in

Switzerland compared to other European countries (Huber *et al.*, 2011; Oppliger *et al.*, 2012; Overesch *et al.*, 2011; Schwendener and Perreten 2011), prevalence of nasally carried MSSA CC398 and other pig associated clonal complex of *S. aureus* (both in pigs and pig farmers) is significant (Oppliger *et al.*, 2012). This study shows also that the genotype composition of *S. aureus* from pigs and farmers was quite similar, implying that MSSA was readily transmitted from pigs to humans. The transmission route of MRSA and MSSA from animals to humans is not well established and bioaerosols could play an important role. An adult inhale 10'000 l of air per day and it is plausible that airborne bacteria perturb the nasal bacterial communities of human (Camarinha-Silva *et al.* 2012). Microorganisms colonizing pigs (from skin, faeces and nostril) can be easily aerosolized in densely populated and enclosed farm buildings. Several studies have demonstrated that organic dust is present in high concentrations and has potential health effects on pig farmers, who can develop a variety of symptoms, including chronic cough, rhinitis, irritation, lung inflammation and decline of lung function (Vogelzang, Schenker *et al.*, 1998, Crook *et al.*, 1991, Nihal Angunna Gamage *et al.*, 2007, Basinas *et al.* 2012, Schwartz *et al.*). To better prevent health concerns in animal confinement buildings, we need to assess more specifically the bacterial composition of airborne dust. It is particularly important to have knowledge of the presence of pathogenic zoonotic strains such as pig-associated *Staphylococcus aureus* and it is important to know whether environmental factors such as season, type of pig farm and size of pig farms can influence the concentration of these bacterial strains.

The aim of this project was to assess airborne concentration of zoonotic MRSA and MSSA in pig farms by using a traditional culture-dependent method and by using molecular quantification of DNA to estimate the proportion of airborne *Staphylococcus sp.* Influence of environmental factors such as season, size and type of pig farms has been performed. Other

important classical airborne contamination indicators (fungi and endotoxins) were measured to allow comparison with other studies.

## **MATERIAL AND METHODS**

### *Study population and sites of sampling*

The study included 37 pig farms, which were spread over the western part of Switzerland. Each of them was investigated once in either 2008 or 2009. We visited 2 or 3 pig farms per day. Pig farms consisted either of nursery and farrowing units where sows give birth and piglets are kept until they weigh about 20 kg (20 farms), or of fattening units where the pigs are kept until they are 90-100 kg (17 farms). The number of pigs per farm varied from 4 to 280, with a mean of 90 pigs in fattening units and 20 sows in nursery/farrowing units. Pigs were kept in boxes (12-20 pigs per box and 2-12 boxes per room) on wooden grating. Sows were kept with their piglets in individual boxes (4-10 boxes per room) directly on concrete. The boxes were equipped with automatic feeding and watering. Most of the buildings had a main passageway with animal boxes on either one or both sides. Most of pig buildings were totally enclosed, and 3 buildings were partially enclosed (pigs having the possibility to go outside). In each pig farm, air was continuously sampled for 3-4 hours during normal occupational activities in the morning hours, at one stationary point in the middle of the passageway at 1.5 m from the floor. At each stationary sampling spot, samples were collected in parallel onto two separate filter devices (one for endotoxins and one for DNA), by means of pocket pumps (MSA Escort Elf, Mine Safety Appliance Company, Pittsburgh, USA or SKC pocket pump 210-1002, SKC Inc., USA), set at a flow rate of 2.0 l/min. Airborne bacteria and fungi were collected in duplicate on nutrient media, at the same stationary point with an impactor (MAS-100 Eco, MBV; Vevey, Switzerland) at a flow rate of 100 l/min.

### *Micro-organisms analysis*

Air volumes of 10 l were sampled for non-specific *Staphylococcus* onto SAID plates (Biomérieux, Marcy-l'Etoile, France), 250 l for MRSA onto MRSA chromogenic plates (Biorad-Laboratories, Cressier, Switzerland) and 50 l for fungi onto Sabouraud (Oxoid, Basel, Switzerland). These different air volumes were chosen to optimize the number of colonies on plates. Plates were incubated at 25°C for fungi and 37°C for bacteria. All plates were daily checked during 3 days to check for specific colonies. Results were expressed in Colony Forming Units (CFU) per cubic meter of air. Suspected *S. aureus* colonies were isolated and subsequently identified by first using an agglutination kit diagnostic test (STAPHAUREX, Oxoid, Basel, Switzerland) and then by molecular identification (see details in Sakwinska *et al.*, 2009).

### *Endotoxin analysis*

Endotoxins were sampled in 30 pig farms and were measured as described in a previous study (Oppliger *et al.*, 2005). Briefly, airborne dust collected onto polycarbonate filters (37 mm diameter, 0.4 µm pore size) placed in a ready to use polystyrene cassette (endofree cassette, Aerotech Laboratories, Inc., Phoenix, USA) was transported within two hours of sampling to the laboratory in a cold box and was then stored at -20°C for 1-3 months to await endotoxin measurement. Endotoxins were extracted by shaking the filters at room temperature for one hour in 10 ml of pyrogen-free water in a 50 ml conical polypropylene tube. The filter extraction solutions were then analyzed with a quantitative kinetic chromogenic LAL (Limulus Ameobocyte Lysat) assay (Biowhittaker, Cambrex Bio Science, Verviers, Belgium)

at 37 °C. *Escherichia coli* O55:B5 endotoxin (Biowhittaker) was used as a calibration standard. Results were expressed in Units of Endotoxin (EU) per cubic meter of air.

#### *Sampling of airborne bacterial DNA and nucleic acid extraction*

Airborne bacterial DNA sample collected onto a 3 µm pore size 25 mm gelatin filter (SKC, Inc. Eighty Four, USA) placed in a button aerosol sampler (SKC, Inc. Eighty Four, USA) at a flow rate of 4 l/min for 4 hours were stored at -20°C until further use. Gelatin filters were dissolved, and treated with lysozyme and proteinase K in 20 µl as described in Verreault *et al.*, (2010). Then, DNA was directly extracted from the lysate with the FastDNA® SPIN Kit for Soil (Bio101, Carlsbad, Calif.). The protocol was slightly modified from the manufacturer's instructions: 650 µl of sodium-phosphate buffer and 80 µl of MT Buffer were added to the lysate, since previous results have demonstrated better recovery of DNA when applying this modification. The sample was processed in a Fastprep® system and centrifuged. The supernatant was recovered in a new tube. The pellet was dissolved in 400 µl of sodium-phosphate buffer and 50 µl of MT Buffer, and then processed a second time in the Fastprep® system to ensure maximal recovery of DNA. Following potassium acetate treatment, the supernatant was treated with 500 µl of Binding Matrix solution and 500 µl of 6M guanidium thiocyanate. Finally, the DNA was eluted from the matrix with 200 µl of ddH<sub>2</sub>O.

#### *Real-time PCR amplification and quantification*

All polymerase chain reaction (PCR) assays were performed on a Rotor-Gene 3000 thermal cycler (Qiagen, Switzerland) and results were analyzed by Rotor-Gene software, version 6.1(93) (Qiagen, Switzerland). All DNA samples were run in triplicate. Highly concentrated DNA samples were tenfold diluted for easier analysis and to assess the presence

of inhibitors. Standards were included in each run. Negative controls with double-distilled water, instead of template DNA, were included in triplicate in each run.

Total bacterial load assessment: A 466-bp fragment of the bacterial 16S rDNA 331-797 (according to *Escherichia coli* position) was amplified with a universal primer and probe set (Nadkarni *et al.*, 2002). The PCR was performed essentially as described in Rinsoz *et al.*, 2008. Quantification was achieved by using calibration curves generated with a serially diluted plasmid.

*Staphylococcus species.* load assessment: A 370-bp fragment of the *Staphylococcus* species *tuf* gene, which encodes the elongation factor Tu, was amplified with a primer set specifically for the *Staphylococcus* genus (Martineau *et al.*, 2001). The PCR was performed in a total volume of 20 µl essentially as described in Rinsoz *et al.*, 2008. Quantification was achieved by using calibration curves generated with a serially diluted plasmid.

Standard curves: The 466-bp PCR fragment of the 16S rDNA and the 370-bp PCR fragment of the *tuf* gene were ligated into a pGEM-T Easy Vector system (Promega). The amount of each extracted plasmid was measured with a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies) and the corresponding copy number was calculated. For each plasmid, a 6-point calibration curve (quantification cycle (C<sub>q</sub>) value versus log of initial plasmid copy number) was generated for qPCR using tenfold serial dilution of the plasmid. Based on the calibration curves, both total bacterial load and staphylococcal load were expressed as gene copy number per unit of volume.

### *Statistical methods*

All bioaerosol data were log-transformed prior to analyses. The influence on bioaerosols of type of pig farm (nursery or fattening), size of pig farm (small with <20 pigs; medium with 20-50 pigs and big with >50 pigs) and season (winter or summer), were tested with an analysis of variance (ANOVA). All statistics were done by using Systat or STATA software

(SYSTAT Software Inc. products Canada; StataCorp, Texas USA). The data are presented by arithmetic mean values and range (min-max). Geometric means are presented only in the table.

## RESULTS

### *Prevalence of airborne MRSA and MSSA*

Airborne MSSA were isolated from 32.4% (12/37) of farms with a mean of 1564 CFU/m<sup>3</sup> air (range 100-4000 CFU/m<sup>3</sup>). They were found significantly more frequently in winter (7/12) than in summer (5/25) (Yates corrected Chi-square = 3.8, p = 0.05). Airborne MRSA CC398 (300 CFU/m<sup>3</sup> air) was discovered on one farm.

### *Effect of season, size and type of production on bioaerosol levels*

Results of annual and seasonal levels of bioaerosols are presented in Table 1. The mean airborne endotoxin concentrations in winter were about 3 times higher than the Swiss recommended limit value of 1000 UE/m<sup>3</sup> (SUVA 2012), whereas the mean summer concentrations were slightly below this threshold. Ninety percent (27/30) of the measured endotoxins sampled exceeded 90 UE/m<sup>3</sup>, which is the recent recommended exposure limit suggested by an expert committee associated with the Health Council of the Netherlands (DECOS, HCN 2010). In winter, mean airborne concentrations of fungi were 15 times higher than the Swiss recommended limit value of 1000 CFU/m<sup>3</sup>. Concerning the DNA copy number of total bacteria and *Staphylococcus sp*, mean airborne concentrations were very high, and DNA copy number of *Staphylococcus sp* represents between 0.005 and 12.8% (with a mean of 2.4%) of the DNA copy number of the total bacteria. Analysis of variance (mixed-effect model) showed that the season had a significant influence on the mean quantity of endotoxins, fungi and DNA copy number of total bacteria and *Staphylococcus sp* with higher level in

winter, while size of pig farm and type of pig production (nursery versus fattening) had no effect (Table 2).

## DISCUSSION

The most important result of this study is the high prevalence of pig farms contaminated with airborne pig associated MSSA. Indeed about one third of the air samples of the investigated farms are contaminated with MSSA. The worrying concern is that a previous study (Oppliger *et al.* 2012) carried out in the same pig farms has demonstrated that MSSA isolated from the nasal swabs of pigs and pig farmers, even if they were sensitive to methicillin, harbored high levels of multi-resistance to other antibiotic drugs, in particular to tetracyclines and macrolides. As instance, among isolates from pig farmers, 52% were resistant to tetracycline, while in persons without contact with farm's animals, no isolates were resistant to tetracycline, an antibiotic used both in veterinary and human medicine. Tetracycline resistance genes have also been found in the nasal flora of pig farmers and in air samples from swine confinement houses in Canada (Létourneau *et al.*, 2010). Thus, potential airborne environmental dissemination of drug resistant bacteria from pig houses concerns not only methicillin resistance genes but also tetracycline resistance genes.

Our results show also for the first time in Switzerland the presence of MRSA CC398 in the air of a pig farm. Only one research group in Germany (Schulz and Hartung 2009; Schulz *et al.*, 2012; Friese *et al.*, 2010) has isolated airborne MRSA associated to the clonal complex 398, in pig fattening units housing pigs with positive nasal carriage of MRSA and a conference report of an agricultural forum in USA signals the presence of MRSA in air samples of pig farms but without details on the prevalence and the genotype of the isolated strains (Harper *et al.*, 2010). In regard to these few studies, we can suppose that airborne pig-associated *S. aureus* (MRSA or and/or MSSA) are present in each pig farm housing pigs colonized with *S. aureus*.

The presence of airborne MRSA and MSSA in pig farms means that *S. aureus* traditional routes of transmission from a colonized carrier to another animal or human could not be restricted to direct contact, as currently accepted. It seems highly likely that *S. aureus* could colonize the human nostrils via simple inhalation of contaminated air. The precise mechanisms whereby *S. aureus* colonizes the nostril are still unknown, but several bacterial surface proteins are implicated in promoting adhesion to desquamated epithelial cells. Competition with other commensal microbes is necessary, as are mechanisms of adhesion, invasion and immune evasion (Edwards et al. 2012). Elimination of the other nasal strains could be favored by the presence of high levels of airborne endotoxins, which are well known for their pro-inflammatory capability. Indeed, pro-inflammatory cytokines released in the upper respiratory tract in response to endotoxin exposure (Wang et al., 1997) could promote elimination of the commensal bacterial strains, thus favoring the settlement of bacteria, such as *S. aureus*, able to escape an immune response. This hypothesis needs further investigations.

Beside *S. aureus*, results showed that other *Staphylococci* species (coagulase negative) are well present in pig farms. But it was found to be impossible to apply standard culture-dependent methods using impaction of bacteria onto nutrient media to enumerate them. Indeed, collection of only 10 l air onto culture media specific for *Staphylococci* resulted in an over-crowded growth (impossible to count) of different phenotypes of *Staphylococcus* species. However, quantification of these strains, by using PCR amplification, showed that they represented only 2.4% of the total bacteria. This is higher than the proportion of 0.8% of *Staphylococci* found in poultry houses with the same qPCR methods (Oppliger et al. 2008).

The number of the DNA copy number of total bacteria is in the same range as the results of a Canadian study (Nehmé et al., 2009) that found between  $10^6$  to  $10^8$  DNA copy numbers of total bacteria/m<sup>3</sup> air in a swine confinement building. Molecular quantification yields higher numbers than culture-dependent methods, which confirm that most of environmental bacteria

are non-cultivable and that one need to use molecular methods to quantify and also to identify bacterial communities in such complex environment.

Fungal exposure is non negligible since about half the pig farms had concentrations above the Swiss recommendation (1000 CFU/m<sup>3</sup>). Exposure to high levels of fungi could be problematic since a lot of species are allergenic and under certain circumstances some species can produce mycotoxins that are toxic for humans and animals health. However, medical and/or epidemiological data on that potential health risks in pigs farmers has never been investigated. Concerning endotoxins, in 36% of the pig farms, levels of airborne endotoxins exceeded the Swiss recommended OEL of 1000 EU/m<sup>3</sup>, and 90% of the pig farms exceeded the “no adverse effects” level of chronic exposure fixed at 90 UE/m<sup>3</sup> by the Dutch Expert Committee on Occupational Safety (DECOS, 2011). A review, which included 27 different studies, shows that endotoxin levels in pig farms were highly variable (Cole *et al.*, 2001) and difficult to compare due to the lack of methodology standardization. However, endotoxin levels found in this study were much lower than values measured during hog load-out and during swine building power washing (O’Shaughnessy *et al.*, 2012), or during handling of other farm animals such as poultry (Oppliger *et al.*, 2008). Chronic exposure to endotoxins can impair respiratory health (Liebers *et al.*, 2006) and it has been shown that endotoxins are a major determinant of lung function decline in pig farmers (Vogelzang *et al.*, 1998). To prevent deleterious and irreversible impairments, lung functions of farmers should be monitored regularly throughout their working life by occupational or general practitioner physicians.

The size and the type of farms (fattening or nursery/farrowing units) have no significant effects on the quantity of bioaerosols. These results were surprising since, due to the increase number of pigs in big farms and the higher density of animals in fattening units than nursery units, we expected to observe a higher bioaerosol exposure in big farms and in fattening units

than in small farms and nursery or farrowing units. It is possible that the limited sample size of the farms investigated did not allow the highlight of these effects. On the other hand, season has a significant influence on the level of airborne MSSA, *Staphylococcus sp*, total bacteria, endotoxins and fungi, with higher levels found in winter. This is in agreement with other studies carried out in pig houses in Canada and the USA (Duchaine *et al.*, 2000; Kiekhaefer *et al.*, 1995). The reduced ventilation in confinement houses to avoid heat loss in winter should be responsible for that increased concentration of organic dust composed mainly of fungi, microorganisms and their by-products such as endotoxins.

In conclusion, we can highlight that Swiss pig farmers are potentially exposed to high quantity of bioaerosols, particularly during winter months and that, like their European neighbours, they are now exposed to a new, emerging, biological occupational risk related specifically to the zoonotic *S. aureus* strain CC398. This strain is, in Switzerland, a common multi resistant colonizer of pigs, which poses a problem of occupational health but also a considerable public health problem. Indeed, airborne bacteria can survive and disseminate over long distances from their emission site (Ko *et al.*, 2008; Yuan *et al.*, 2010, Schulz *et al.*, 2011) and therefore could be dispersed and colonized new human or animal hosts living close to the pig houses.

In order to avoid transmission of *S. aureus* from animals to humans, very strict hygiene measures should be recommended (hand washing, using piggery-specific work clothing and using a changing room next to the pig buildings, taking a shower at the end of work shifts, careful disinfection and protection of any small cuts or grazes on the skin), as well as using gloves when handling animals themselves and wearing a respirator mask (P2 type) when carrying out activities that generate a large amount of dust.

Pig farmers should be advised that in case of hospitalization, it is important to inform their doctors that they work with animals and could be carriers of “pig-associated *S. aureus*”.

Although *S. aureus* generally poses little risk to a person in good health, the nasal carriage upon hospitalization is seen as a definitive risk factor for surgical wound infection, and bloodstream infections in immunocompromised patients (i.e. Bode *et al.*, 2010).

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Table 1: Arithmetic mean /*geometric mean*, range (min – max) and sample size of the different bioaerosols measured in pig farms in Switzerland.

Parameters	Total mean	Summer mean	Winter mean
Cultivable <i>S. aureus</i> CFU/m <sup>3</sup> *	1616 / 1011 (100 – 4000) N = 12	580 / 323 (100 – 1700) N = 5	2357 / 2279 (2000 – 4000) N = 7
DNA copy number of Staphylococcus sp/m <sup>3</sup>	$1.6 \times 10^7 / 8.6 \times 10^5$ ( $2 \times 10^3 - 4 \times 10^8$ ) N = 34	$3.6 \times 10^6 / 4.3 \times 10^5$ ( $1.9 \times 10^3 - 4.7 \times 10^7$ ) N = 23	$4.1 \times 10^7 / 3.5 \times 10^6$ ( $5.9 \times 10^4 - 4 \times 10^8$ ) N = 11
DNA copy number of total bacteria/m <sup>3</sup>	$3.6 \times 10^9 / 7.7 \times 10^7$ ( $1.6 \times 10^5 - 6.1 \times 10^{10}$ ) N = 34	$7.5 \times 10^8 / 3.5 \times 10^7$ ( $6.1 \times 10^4 - 1.4 \times 10^{10}$ ) N = 23	$9.6 \times 10^9 / 4.1 \times 10^8$ ( $1.5 \times 10^6 - 6.1 \times 10^{10}$ ) N = 11
Fungi CFU/m <sup>3</sup>	5707 / 770 (20 – 52560) N = 37	1049 / 404 (20 – 5370) N = 25	15412 / 2943 (30 – 52560) N = 12
Endotoxin EU/m <sup>3</sup>	1289 / 636 (17 – 6149) N = 30	798 / 444 (17 – 2678) N = 24	3253 / 2666 (721 – 6149) N = 6

\*37 pig farms sampled but mean calculated only with the positive farms

Table 2: Relative effect (Coef. = coefficient) F and P value of season, size and type of pig farms on bioaerosols concentrations, in Swiss pig farms. Mean values of each season for each bioaerosol are presented in the Table 1. \* = statistically significant

		Endotoxin N = 30			Cultivable fungi N = 37			DNA copy nb of total bacteria N = 34			DNA copy nb of <i>Staphylococcus sp</i> N = 34		
		Coef.	F	P	Coef.	F	P	Coef.	F	P	Coef.	F	P
Season	summer (ref)	0	5.02	<b>0.03*</b>	0	9.67	<b>0.004*</b>	0	4.96	<b>0.03*</b>	0	6.97	<b>0.01*</b>
	winter	1.38			2.09			2.68			2.24		
Size	small (ref)	0	0.97	0.39	0	3.04	0.06	0	0.79	0.46	0	1.60	0.21
	medium	-0.16			1.69			-1.81			-1.81		
	big	0.63			0.34			-1.63			-1.70		
Type	nursery (ref)	0	0.18	0.67	0	0.79	0.38	0	0.39	0.54	0	0.99	0.32
	fattening	0.24			-0.64			0.80			-0.90		
Intercept		5.94			5.42			18.2			14.57		