A SURVEY OF ZOONOTIC AGENTS IN ITALIAN RABBIT SLAUGHTERHOUSES

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ABSTRACT

From June 2002 to February 2003 five rabbit slaughterhouses were selected in Campania region (Italy) on the basis of their geographic location and their slaughtering capacity. Samples from muscular tissue, carcass swabs, organs and carcass washing water were collected and examined for *Salmonella spp., Lysteria monocytogenes, Campylobacter spp., Yersinia enterocolitica*; surface air system was used for *Dermatophytes* and hygienic indicators of the slaughtering process were evaluated with the enumeration of coagulase-positive *Staphylococci*, of *E. coli* and of total aerobic mesophylic count. Three different serotypes were respectively detected each one in three of the five monitored slaughterhouses: *S. Indiana* (from carcass washing water), *S. blockley* and a non-typifiable strain (in muscular tissue). Three different strains of *Listeria monocytogenes* were detected in two of the five processing plants; in particular two strains were isolated from muscular tissue and one strain from organs. All the rabbit carcasses were negative for *Campylobacter spp.* and *Yersinia enterocolitica*. Environmental monitoring and hygienic control have shown the presence of dermatophytes above all during stunning (< 42 CFU/m^3), skinning (< 32 CFU/m^3), evisceration (< 30 CFU/m^3) and packing phase (< 28 CFU/m^3) that, together with other hygienic indicators and, particularly, the enumeration of *E. coli* signal the necessity of an increasing attention to the implementation of codes of good manufacturing practice (GMP). Greater care during evisceration and accurate meat inspection procedures should be encouraged to prevent secondary contamination from environment of the slaughtering plant.

Key words: zoonosis**,** rabbit carcasses, slaughter, contamination.

INTRODUCTION

Zoonosis, such as dermatomycosis, are occupational diseases for breeders, veterinarians and slaughtering technicians. Salmonellosis, Listeriosis, Campylobacterioris and other zoonosis are foodborne infections spreading from animals to man (GANIÈRE *et al.* 2001). In fact, bacteria reaching man through food of animal origin represent one of the direct causes of this sort of diseases (SCHLEGELOVÁ *et al.* 2004). Sources of contamination during slaughtering are both environmental, and animal related, such as through faeces. During the evisceration step the spread of zoonotic bacteria out of carcass surface is mainly due to the presence of carrier animal and, therefore, in the slaughterhouses, good hygienic practices and equipment cleanliness are relevant both to prevent and to reduce the level of carcass contamination (BONARDI *et al.* 2003). In fact the contamination with strains of *Staphylococcus* and *E. coli* is often caused by contaminated skin and water, by faeces and contents of digestive organs. If the contamination of the surface of meat occurs immediately after evisceration, it might be due to strains coming directly from animals; if it occurs after cooling, the carriers might be animals, people and equipment (SCHLEGELOVÁ *et al.* 2004). Zoonosis monitoring is particularly important in a productive and legal frame, also to collect epidemiological data about production chains in as much as the zoonosis epidemiological risk assessment requires an integration between the traditional clinical approach to rabbit pathology and the hygienic one (FACCHIN *et al*. 1996).
Three were the objectives of the present investigation. The first one was to assess the occurrence of *Salmonella spp, Listeria monocytogenes, Campylobacter spp., Yersinia enterocolitica* together with the risk for contamination of carcasses, during the compulsory meat inspection procedures and the controls of slaughtering process in five rabbit slaughterhouses located in Campania region (Italy). The second purpose was to identify the possible occurrences of meat contamination and its risk for consumers, and the third aim was improvement of diagnostic tools for effective monitoring.

MATERIALS AND METHODS

Collection of samples

During June 2002 and February 2003 five rabbit slaughterhouses (A, B, C, D, E) located in Campania region (Italy) were inspected. The plants were selected on the basis of their location (capital of a province), of their structural organization and slaughtering capacity (500-1,000 animals/day; 1,000-3,000 animals/day). For each processing plant, four supplying breeding farms were chosen according to the dimension of their slaughtering batch (i.e. supplied rabbits almost equal to the slaughtering capacity of the plant) and business frequency. This condition enabled a control of at least two batches per supplier (Table 1).

The following samples were collected from each batch: a) 3 samples of muscular tissue from each carcass just after evisceration (part 1), at the end of the cooling tunnel (part 2), and after the packing (part 3); b) one 100 cm^2 swab from the shoulder after the packing; c) samples of organs (cholecyst); d) carcass washing water; e) two “surfair

plates" (Surface Air System) to evaluate the presence of *Dermatophytes.* Table 2 shows the analysis carried out and the investigated strains.

Table 1. Typology and number of samples

Average number of slaughtered rabbits related to slaughtering capacity	*Monitored supplying breeding farms*	*Monitored batches*	*Total slaughtered rabbits*	*Carcasses for Pool part 1, 2, 3*	*Cholecysts*	*Carcasses used for enumerations*	*Swabs*	*Carcass washing water*	*Surfair plates*			
				Collected samples: Total number					***Stunning***	***Skinning***	***Evisceration***	***Packing***
A) AVELLINO 3,000/day **2,000 –3,000 animals**	**3**	**7**	8,350	84	560	42	42	7	*14*	*14*	*14*	*14*
B) Benevento 2,000/day **1,000 –2,000 animals**	**5**	**9**	17,900	81	360	36	36	9	*18*	*18*	*18*	*18*
C) CASERTA 1,500/DAY 1,000 –2,000 animals	**5**	**10**	7,247	90	400	40	40	10	*20*	*20*	*20*	*20*
D) NAPLES 600/DAY 500 –1,000 animals	**5**	**9**	5,100	54	180	27	27	9	*18*	*18*	*18*	*18*
E) SALERNO 300/DAY 500 –1,000 animals	**3**	**10**	3,200	60	200	30	30	10	*20*	*20*	*20*	*20*
TOTAL	**21**	**45**	**41,797**	**369**	**1,700**	**175**	**175**	**45**	**90**	**90**	**90**	**90**

Table 2. Analysis of samples and investigated strains

CARCASS SWABS (100 CM²) **AFTER PACKING**	**POOL OF CHOLECYST** **1 cholecyst/15 carcasses/batch** (Salerno and Naples), **1 cholecyst/50 carcasses/batch** (Caserta and Benevento) , **1 cholecyst/35 carcasses/batch (Avellino)**	**6 carcasses/batch** (Salerno and Naples), **9 carcasses/batch** (Caserta and Benevento), **12 carcasses/batch** (Avellino)		
		PART 1 (AFTER EVISCERATION)	**PART 2 (AFTER COOLING)**	**PART 3 (AFTER PACKING)**
1) ENUMERATION OF COAGULASE-POSITIVE STAPHYLOCOCCUS 2) TOTAL AEROBIC MESOPHILIC COUNT	*1) SALMONELLA SPP, 2) L. MONOCYTOGENES, 3) CAMPYLOBACTER SPP, 4) Y. ENTEROCOLITICA*	*1) SALMONELLA SPP, 2) L. MONOCYTOGENES, 3) CAMPYLOBACTER SPP , 4) Y. ENTEROCOLITICA 5) ENUMERATION OF E. COLI*	*1) SALMONELLA SPP, 2) L. MONOCYTOGENES, 3) CAMPYLOBACTER SPP, 4) Y. ENTEROCOLITICA 5) ENUMERATION OF E. COLI 6) TOTAL AEROBIC MESOPHILIC COUNT*	*1) SALMONELLA SPP , 2) L. MONOCYTOGENES, 3) CAMPYLOBACTER SPP, 4) Y. ENTEROCOLITICA 5) ENUMERATION OF E. COLI 6) TOTAL AEROBIC MESOPHILIC COUNT 7) ENUMERATION OF COAGULASE-POSITIVE STAPHYLOCOCCUS*
Carcass washing water in evisceration site –1 litre/batch – *SALMONELLA SPP*				
Mycological analysis (*MYCROSPORUM***,** *TRYCOPHYTON***) -8 surfair plates/batch** (stunning, skinning, evisceration and packing sites)				

Isolation and detection

The samples were transported at 4 °C to the laboratory. Upon arrival at the laboratory, all the samples were either immediately analysed, or were held at 4 °C for no longer than 24 h before analysis.
Samples were analysed by conventional methods for food microbiology (Rapporto ISTISAN 96/35, AFNOR V08-053/1993); Surface Air System (SAS) and surfair plates with Mycobiotic Agar (Difco) were used to detect dermatophytes.

RESULTS AND DISCUSSION

Results of microbiological examinations of water samples, swabs and muscular tissue are summarised in Tables 3 - 4 and Figures 1 – 8.
Salmonella spp. was isolated from three of 45 total batches investigated: *S. blockley* and another non-typifiable strain were isolated from meat and *S. indiana* from carcass

washing water. *S. blockley* is a not specific host serotype. It can be isolated from human, animal, food and environmental sources, and the serotype is characterized by a multiresistance to antimicrobial drugs (FISICHELLA *et al.* 2003). These data confirm the hypothesis that in rabbit slaughterhouses the isolation of *Salmonella spp.* is also possible from transport cages and processing plant cross contamination. The low incidence of Salmonellosis in commercial rabbitries does not imply the possibility of a decreasing attention to this zoonosis; but it calls for an accurate checking system to involve all batches sent to slaughtering plants (ZANON *et al.* 1996). Three strains of *L. monocytogenes* were isolated from organs and meat of two batches in the same slaughter, where other five batches were positive for *L. innocua* and *L. grayi*. Epidemiological studies about the presence of *Listeria* in human food showed the high presence of *L. innocua,* a species which is not pathogenic and not haemolytic. *L. monocytogenes* in foodstuffs is a latent risk of Listeriosis (BOSGIRAUD *et al.* 1991), even if it is not well known which is the minimum number of pathogenic *L. monocytogenes* cells that should be ingested to cause illness in either normal or susceptible individuals (FARBER, PETERKIN 1991). *L. monocytogenes* is ubiquitous but in commercial farms it seldom causes illness in rabbits during their productive life-time. Small units, however, are more susceptible. The rabbit's role in the transmission of this pathogen is unknown, but its involvement is highly unlikely (OKERMAN 1994). All the rabbit carcasses investigated were negative for *Campylobacter spp.* and *Yersinia enterocolitica* isolation.
Environmental monitoring and hygienic control have shown the presence of dermatophytes in all slaughterhouses, above all during the following phases: stunning (< 42 CFU/m^3), skinning (< 32 CFU/m^3), evisceration (< 30 CFU/m^3) and packing (< 28 CFU/m^3). The morphological identification (macroscopic and microscopic) isolated 32 strains; *Microsporum canis* was found in 56.3 % of the cases (18/32), *Trichophyton mentagrophytes* in 31.2 % of the cases (10/32) and *Microsporum gypseum* in 12.5 % of the cases (4/32). These data, together with other hygienic indicators and, particularly, the enumeration of *E. coli* signal the necessity of an increasing attention to the implementation of codes of good manufacturing practice (GMP).

Table 3. Results of microbiological examination – Zoonotic bacteria

RABBITRIES LOCATION	Nº POSITIVE BATCHES No. of positive batches/ No. examined batches	POOL OF CHOLECYST	POOL PART 1	POOL PART 2	POOL PART 3
A) AVELLINO	0	------	----------	----------	----------
B) BENEVENTO	1/9	***L. monocytogenes***	***S. blockley 6,8; K; 1,5***	***S. blockley 6,8; K; 1,5***	***L.monocytogenes***
C) CASERTA	7/10	***L. innocua*** ***L. grayi***			***L. grayi*** ***L.innocua***
				L. grayi	***L. monocytogenes***
D) NAPLES	1/9			***L. grayi***	
E) SALERNO	1/10			***Salmonella spp.***	
Carcass washing water in evisceration site - 1 positive batch – Caserta (*Salmonella indiana* B; Z; 1,7)					

CONCLUSIONS

The results confirm the microbiological quality of rabbit meat if compared to other species. It is well considered for its nutritive and dietetical value and for its safety, which derive from the absence or a rare presence of zoonotic agents (FACCHIN *et al.* 1998). For an effective control of zoonosis risk it is necessary a monitoring activity of all rabbit meat productive chain (ZANON *et al.* 1998) beginning from the breeding farms. This should be the first step to eliminate most of risk factors for rabbit meat consumers (FACCHIN *et al.* 1998; TANTINÀ *et al.* 2000). On this line a severe selection of rabbit suppliers is necessary to have animals which do not carry pathogenic agents. In order to reduce the risk represented by zoonotic agents to consumer health, it is essential to avoid any contamination by human pathogens during slaughtering. Therefore, some preventive measures, such as implementation of codes of good manufacturing practices, increased care during evisceration, and improvement of the meat inspection procedures, should be encouraged (BONARDI *et al.* 2003).

Table 4. Results of microbiological examination – Hygienic indicators

RANGE SWABS CFU/cm² PART CFU/g	SWABS No. of samples/ 175 total examined samples (%)		No. of samples referred to range/175 total samples examined (%)					
			PART 1	PART 2		PART 3		
	Total aerobic mesophylic count	Coagulase-positive Staphylococcus	E. coli	Total aerobic mesophylic count	E. coli	Total aerobic mesophylic count	E. coli	Coagulase-positive Staphylococcus
0-99			59.43		58.86		61.72	
100-1000			15.43		17.72		17.72	
1001-10000			14.29		11.42		10.85	
> 10000			10.85		12		9.71	
< 3.5 log	87.43			46.29		57.15		
3.5>log <5	5.72			33.71		24		
> 5 log	6.85			20		18.85		
0-100		94.86						86.86
100-1000		5.14						6.86
>1000		0						6.28

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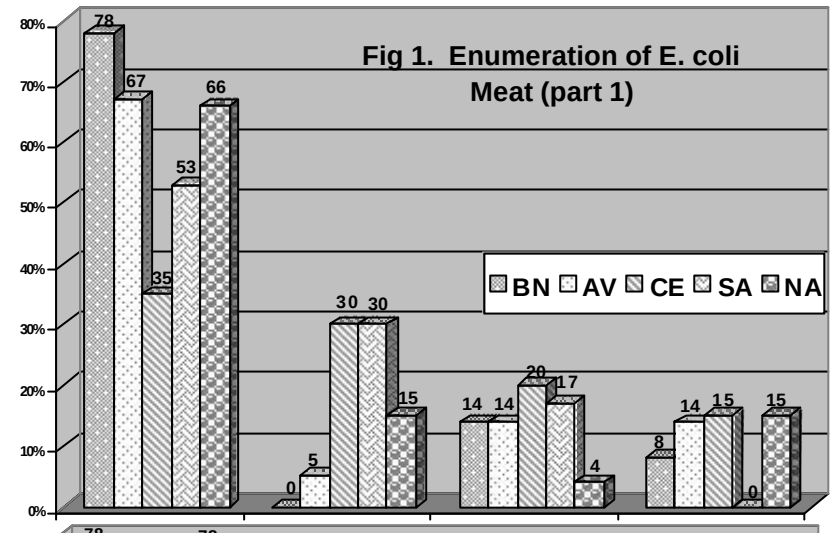
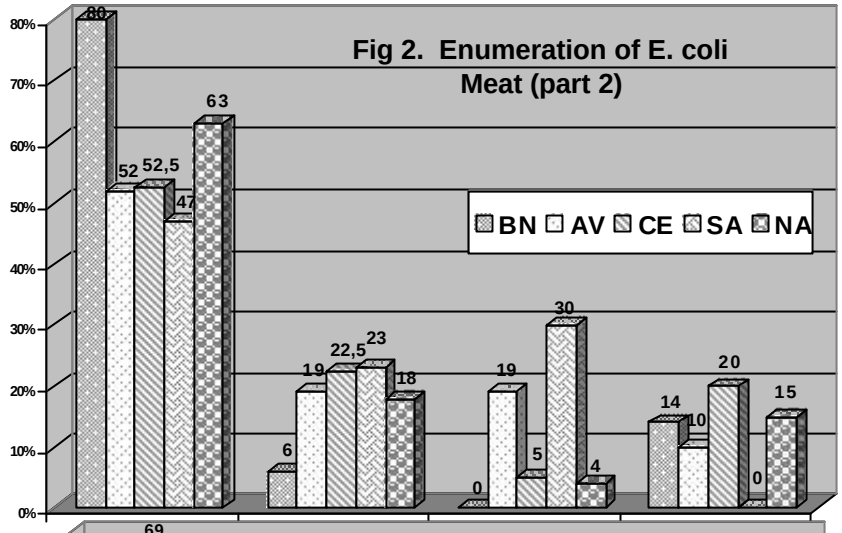
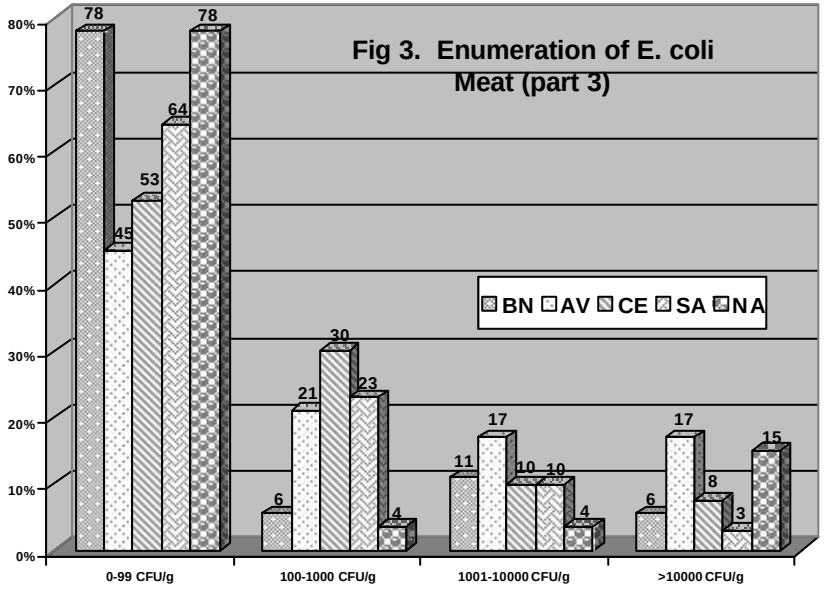






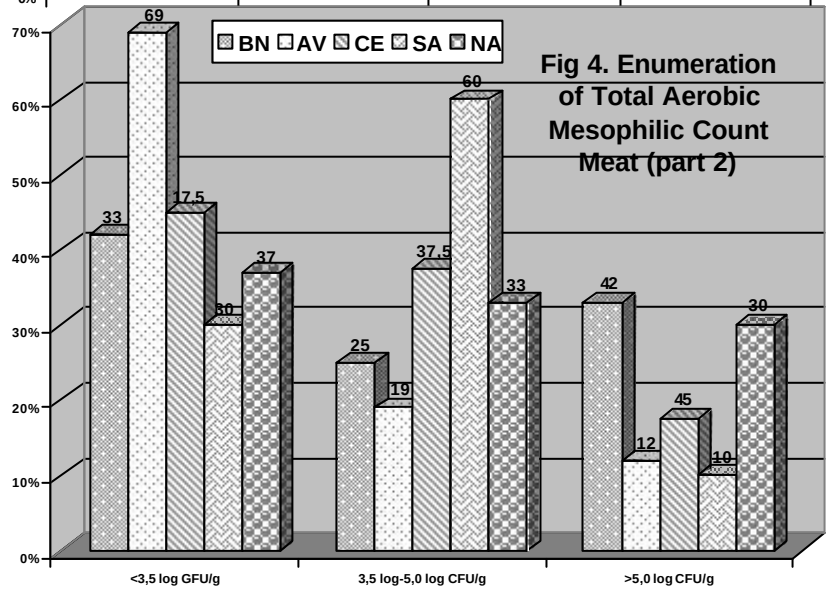
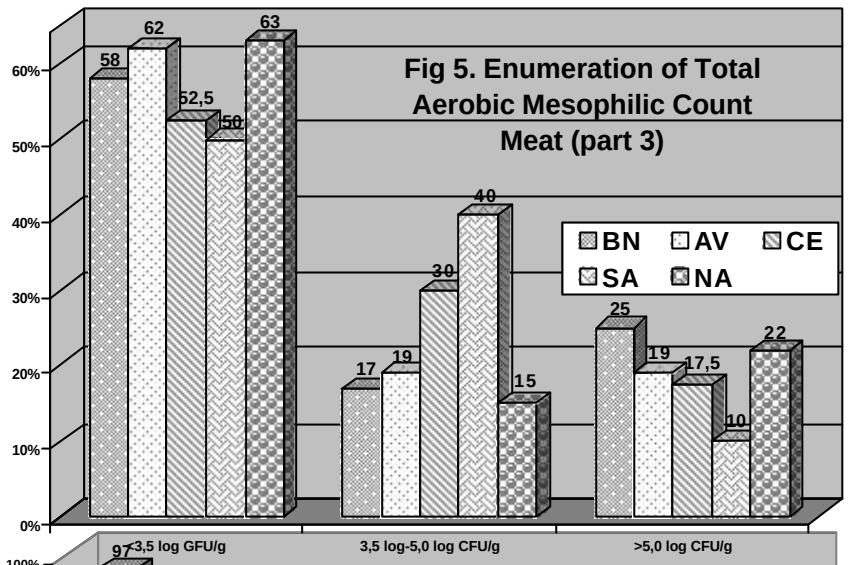
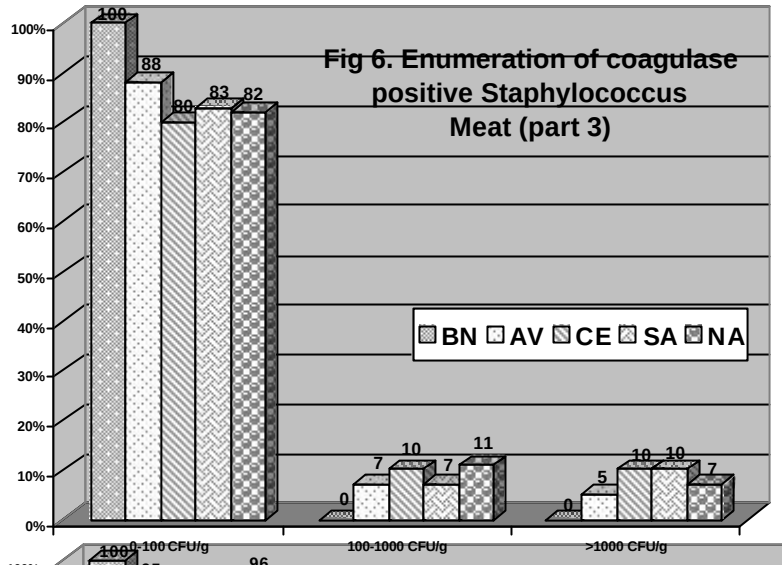
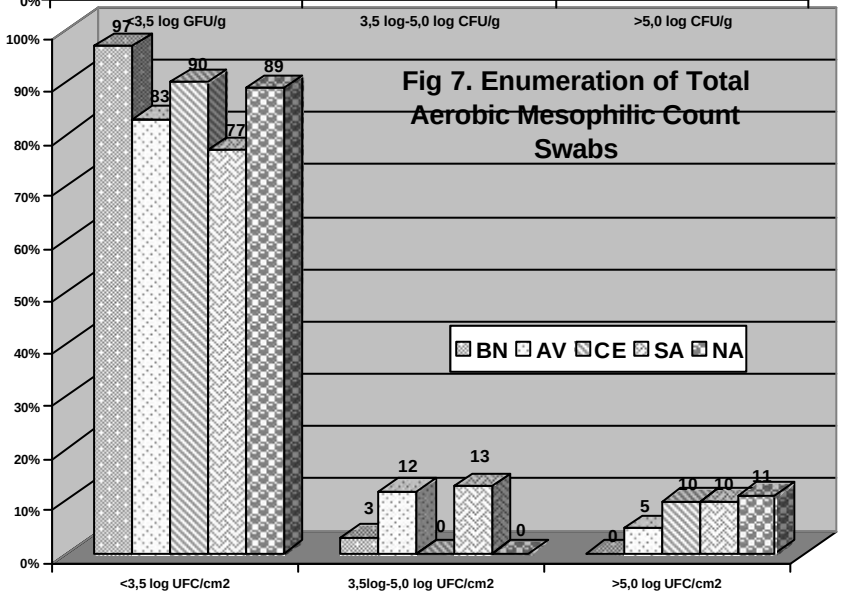


Fig 1-4. Hygienic indicators in slaughtering plants located in Benevento (BN), Avellino (AV), Salerno (SA), Caserta (CE), Naples (NA): range and number of samples (%)







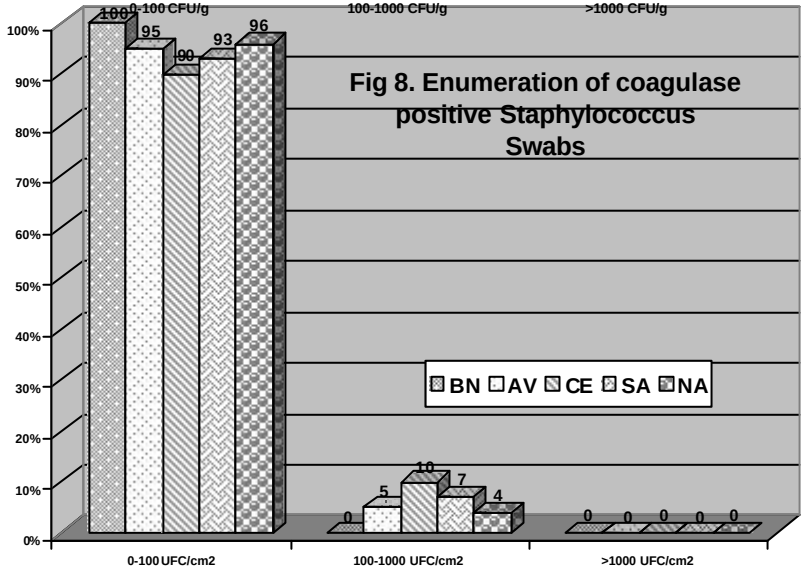


Fig 5-8. Hygienic indicators in slaughtering plants located in Benevento (BN), Avellino (AV), Salerno (SA), Caserta (CE), Naples (NA): range and number of samples (%).