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Microbiological Testing of Surfaces of Food Establishments in Cyprus.

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Sampling and Testing was done
by EPTA Laboratories during
the period between January and
August 2007

PURPOSE OF SURVEILLANCE

- To assess hygiene and efficiency of cleaning and disinfection methods as a part of the internal audit of the Food Establishments.
- To prevent contamination of foods and safeguard food safety.
- To trace the sources and type of microbiological contamination.
- To improve cleaning procedures.
- To increase the shelf life of foods produced
- To comply with legal requirements

SOURCES OF MICROBIAL CONTAMINATION OF SURFACES IN FOOD ESTABLISHMENTS

- Raw Materials
- Air – dust
- Personnel
- Process equipment
- Water
- Insects – flies – cockroaches
- Rodents, mice – rats
- Drainage system

TYPE AND NUMBERS OF FOOD ESTABLISHMENTS IN SURVEILLANCE

- 12 Slaughter houses (Poultry, Sheep, goats, pigs)
- 16 Cutting Plants – red and white meat
- 10 Butcheries
- 10 Restaurants and Confectioneries
- 8 Egg grading and Packing Stations
- 3 Hatcheries

E.U. LEGISLATION ON MICROBIAL TESTING OF SURFACES

- E.U. Regulation 1441/2007
- E.U. Regulations 852/2004, 853/2004, 854/2005
- E.U. Directive 64/433/E.U.
- E.U. Directive 71/118/E.U.
- E.U. Directive 95/23
- E.U. Directive 98/37/E.U.
- Commission Decision 2001/471/EU
- Standard EN 1672 – 2:1997
- Guidelines of the European Hygiene Engineering and Design EHEDG

LEGISLATION OF CYPRUS ON MICROBIAL TESTING OF SURFACES

- Meat Hygiene Law 2003
- Κ.Δ.Π. 776/2003 Regulations for Slaughter Houses and Meat Cutting Plants

PROVISIONS OF CYPRUS LEGISLATION

- Evaluation of the microbial Contamination of Carcasses
- Methods of Sampling
 - Destructive method collection of four pieces of tissue of a total surface of 20cm²
 - Swabbing with sterile swabs made of cotton
 - Contact agar

Points of Sampling of Cattle Carcasses

- Rump
- Flank
- Brisket
- Neck

Points of Sampling of Pig Carcasses

- Ham
- Back
- Belly
- Jowl

Points of Sampling of Sheep and Goat Carcasses

- Flank
- Thorax
- Sternum
- Point of Slaughter

NUMBER OF SAMPLES FROM CARCASSES

- From 5-10 Carcasses once a week
- All Samples must be taken on the same day
- Frequency of Sampling can be reduced to once every fortnight if the results for six consecutive weeks are satisfactory
- The day of sampling must be different every week
- In small slaughterhouses the frequency of carcasses sampling is at the discretion of the Official Veterinarian

METHODS OF BACTERIOLOGICAL EXAMINATION OF SAMPLES

- Samples were transported and kept under refrigeration
Temperature <40C
- Samples were homogenised in a plastic bag for at least two minutes while 100ml diluent was added – Diluent Peptone 0.1% + NaCl 0.85%
- All samples were cultured within 24 hrs after sampling
- Cultures were carried out for the estimation of Total Bacterial Count and for E.Coli counts

BACTERIOLOGICAL CRITERIA FOR SURFACES OF CARCASSES

- All results were classified in three categories
 - a. Acceptable
 - b. Borderline
 - c. Unacceptable

	Acceptable		Borderline >m ≤m	Unacceptable >M
	Cattle Sheep Goats	Pigs	Cattle, Pigs, Sheep Goats	Cattle, Pigs, Sheep Goats
Total bacterial Count	<3.5log	<4.0log	<3.5log <4.0log Pigs <4log <5log	>5 log
Enterobacteriaceae	<1.5log	<2log	<1.5log <2.0log Pigs <2.5log <3.0log	>2.5log Pigs >3log

It is noted that the method of sampling of carcasses surfaces reveals only 20% of the Total Bacterial Count present

PROVISIONS OF THE CYPRUS LEGISLATION FOR MICROBIOLOGICAL SAMPLING FROM SURFACES IN SLAUGHTERHOUSES AND MEAT CUTTING PLANTS

- Sampling of surfaces is obligatory from all processing rooms related to hygiene of the end product
- Methods of Sampling
 - a. Contact agar – ISO method
 - b. Contact VRBG agar – ISO method
- Contact surface of agar plates 20cm²
 - c. Wet cotton swabs with one ml diluent of NaCl – peptone 0.1% agar from a surface of 20cm² marked with a sterile frame, if sampling is done after cleaning and disinfection the diluent for wetting the cotton swabs must contain 30g/l Tween 80 and 3g/l lecithine.
- For sampling wet surfaces dry cotton swabs are satisfactory
- Swabs are collected in vials with 40ml diluent of peptone with 0.1% NaCl agar
- Transport and Storage of samples at 4°C

FREQUENCY OF SAMPLING AND POINT OF SAMPLING – PROVISION OF LEGISLATION

- 10-30 samples every fortnight.
- If the bacteriological results are satisfactory for a long time the frequency of sampling may be reduced by the Official Veterinary Surgeon in charge of the Food Establishment
- Two thirds of the samples must be from surfaces which come in contact with the foods. Other samples from walls, doors, equipment, knives, aprons, water baths for utensils etc
- All bacteriological results must be recorded and presented in columns which will clearly show the hygiene level of the Establishment

BACTERIOLOGICAL METHODS FOR CULTURES – PROVISION OF LEGISLATION

- Apart from those described in the Regulations, ISO methods can be used.
- The results of the examinations must be presented in numbers per cm²
- Incubation period 24 hrs at 37°C±1°C for the total aerobic count
- For the Total Count of Enterobacteriaceae VRBG agar must be used
- Incubation must start within two hours after sampling
- Duration of incubation 24 hrs at 37°C±1°C

EVALUATION OF THE TOTAL BACTERIAL COUNT RESULTS

	Acceptable	Unacceptable
Total Bacterial Count per cm ²	0-10/ cm ²	>10/ cm ²
Enterobacteriaceae	0-1/ cm ²	>1/ cm ²

- All results were notified to the personnel of the establishment.
- All cases of unacceptable results were investigated and discussed with the personnel in charge of cleaning
- Unacceptable results were due to:
 - Inadequate training of personnel
 - Inadequate directions for the use of detergents and disinfectants
 - The use of unsuitable cleaning materials
 - No regular maintenance of equipment used.
 - No supervision of personnel during cleaning

SAMPLING METHODS USED BY EPTA LABORATORIES FOR SURFACES IN FOOD ESTABLISHMENTS

- Swabbing-sterilized cotton swabs were used and as a diluent for dry surfaces sterile 0.85% NaCl + 0.1 peptone agar
- Contact plates with common agar or VRBG agar
- Hygicult (Orion Diagnostica Oy Espoo, Finland, agar slides for bacterial count and Enterobacteriaceae

CLEANING AND DISINFECTION PROCEDURES

- All establishments used detergents and disinfectants supplied by reputable companies from EU countries.
- The standard steps followed were:
 - Pre-rinse
 - Clean
 - Rinse
 - Disinfect
 - Rinse
- Training of cleaning personnel was limited.
- Instructions for the correct use of detergents and disinfectants were not given in the mother language of users
- Use of water was limited due to shortage. Occasionally water from boreholes was used.

TYPE OF SURFACES FROM WHICH SAMPLES WERE TESTED

- Working surfaces which come in contact with raw materials and foods during processing
- Cold Stores – Walls
- Process equipment – Filing – Packing
- Knives
- Hooks
- Balances
- Slicing and Cutting Equipment
- Walls
- Doors

NUMBER OF SAMPLES TESTED BY TYPE OF ESTABLISHMENT

– 12 Slaughter houses	250 samples
– 16 Meat Cutting Plants	180 samples
– 10 Butcheries	125 samples
– 10 Restaurants and Confectioneries	150 samples
– 8 Egg grading and Packing Stations	120 samples
– 3 Hatcheries	150 samples

Total number of Samples examined : 855

Type of Establishment	Samples Tested	Total Bacterial Count					Enterobacteriaceae				
		Acceptable		Unacceptable			Acceptable		Unacceptable		
		No of Samples	Cfu/cm ²	No of Establishments	No of Samples	Cfu/cm ²	No of Samples	Cfu/cm ²	No of Establishments	No of Samples	Cfu/cm ²
Butcheries	125	111	6-10	4	14	10-18	117	0	3	8	2-4
Restaurants – Confectioneries	150	150	0-8	Nil	Nil	Nil	150	0	Nil	Nil	Nil
Egg Grading and Packing Stations	120	96	0-8		24	11-16 16-28	97	0-1	6	23	2-12
Hatcheries	150	145	0-7	1	5	>10	146	0	1	4	>1
Slaughter houses	250	84	0-5	12	148	5-10 10-25	84	0		118	1-2 3-8
Meat Cutting Plants	180	60	0-5	16	65	5-10	180	0	16		

PATHOGENIC AND POTENTIALLY PATHOGENIC BACTERIA ISOLATED FROM SURFACES OF FOOD ESTABLISHMENTS

- Salmonella s.p.p. were isolated from 23 Food Establishments
- Listeria s.p.p., Listeria Innocua and Listeria Welchimeri were isolated from 46 Food Establishments
- Coliforms were isolated from all Establishments tested.
Total Number 59

BACTEROLOGICAL QUALITY OF WATER SUPPLY – NUMBER OF SAMPLES TESTED

- Origin and number of samples
 - Slaughter Houses 54 samples
 - Meat cutting plants 92 samples
 - Butcheries 30 samples
 - Restaurants – Hotels 60 samples
 - Egg Grading and Packing Stations 8 samples
 - Hatcheries 6 samples
- Total Number of Samples Examined: 250

MICROBIOLOGICAL TESTS FOR WATER

- Total Bacterial Count at 37°C
- Escherichia Coli
- Faecal Coliforms
- Enterococci

RESULTS OF MICROBIOLOGICAL TESTING OF SWABS FROM HANDS OF FOOD HANDERS

Establishments	No of	No of Samples	Enterobacteriaceae	Haematolytic Staphylococci
Slaughter houses	12	20	12	6
Meat Cutting	16	34	24	5
Butcherries	10	20	12	4

RESULTS OF MICROBIOLOGICAL TESTING OF 250 SAMPLES OF WATER

Establishments	Micro- biological Parameters Directive 98/83/EU	Acceptable Samples	Unacceptable Samples
Total Bacterial Count at 37°C	20/100ml	250	0
Escherichia Coli	0/250ml	227	33
Faecal Coliforms	0/100ml	232	18
Enterococci	1/100ml	235	15

CONCLUSIONS - RECOMENDATIONS

1. Continue and increase frequency of sampling and testing surfaces of Food Establishments.
2. Improvement of cleaning and disinfection programs.
3. Training of cleaning personnel and close supervision.
4. Training of food handlers on personal hygiene.
5. Use only drinking water for cleaning.
6. Assessment of process hygiene.
7. Research in cooperation with the food industry to identify problems in cleaning and the provision of practical solutions.
8. Resistant strains of *Listeria* and *Salmonellae* to disinfectants may exist.
9. Monitoring for residues of disinfectants and resistant strains of bacteria.
10. Immediate measures to minimize the presence of pathogens and improvement of hygiene.