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ASSESSMENT OF SMALL-SCALE BUFFALO MILK DAIRY PRODUCTION – A PREMISE FOR A DURABLE DEVELOPMENT

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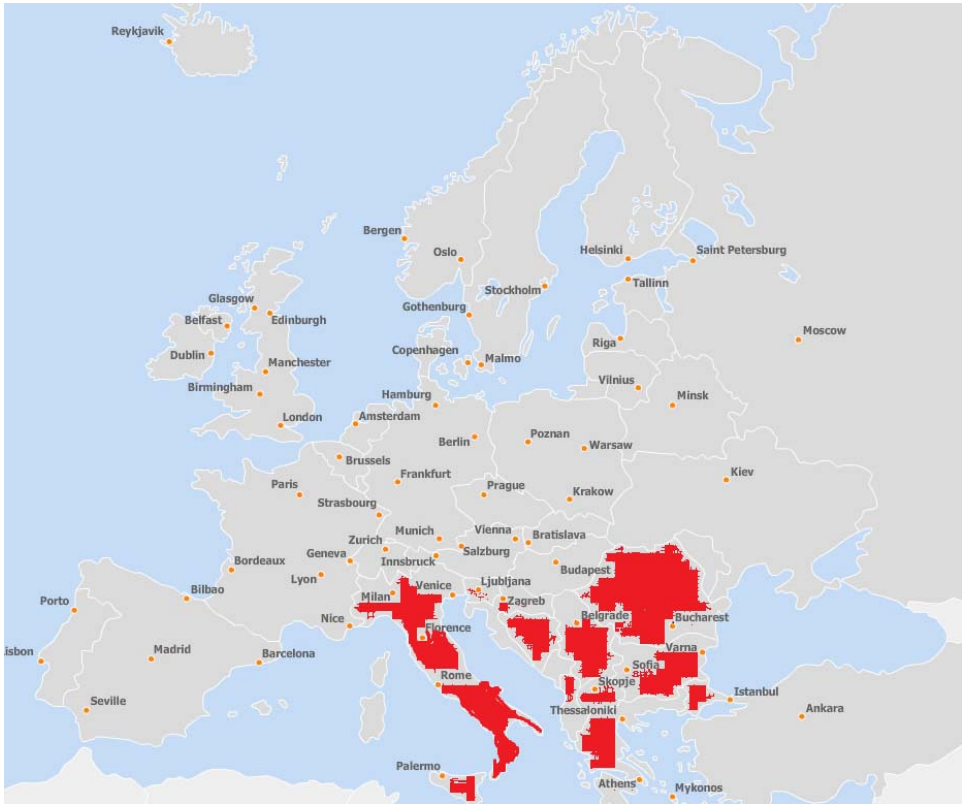
CURRENT SITUATION

The buffalo population in the Mediterranean area (Europe and the countries of the Near East) is about 5.5 million head, 3.4 % of the world buffalo population.



Continuous decrease in number

- Holsteinization: the substitution of low production cows and buffaloes with high production Holstein Friesian cows;
- Mechanization: the substitution of draught animals with tractors;
- Poor market demand for buffalo products.

CURRENT SITUATION IN ROMANIA

A small nucleus is recorded (0.7 percent total buffalo-cows)

Mediterranean breed - sometimes crossbred with Bulgarian Murrah

Buffaloes are raised mostly in small private farms for own consumption or for processing into products destined for the public market.

IMPORTANT ASPECTS OF BUFFALO DAIRY PRODUCTS:

- Lower cholesterol content;
- More proteins: is a more economical alternative to cow milk for production of casein and whey protein concentrates;
- Fat: 40 to 50% higher in buffalo milk than in cow milk;
- Calcium, iron, phosphorus: Ca higher by 92%, iron 37% and P by 118% in buffalo milk than in cow milk;
- Buffalo milk more resistant against oxidative changes;
- Commercially more viable: due to lower water content and higher fat content;
- Because of richness, buffalo milk more suitable for processing;
- More expensive than cow milk.

RESEARCH MOTIVATION:

- ❖ The new social-economical context;
- ❖ The EU quality requirements stated in the legislation;
- ❖ The lack of an accurate and detailed study on the major adulterations, like: forgeries (admixtures with cow milk) and hygiene characterization of these traditional buffalo products found on the Romanian market;
- ❖ The lack of a risk analysis based on accurate data and involving high class techniques.

AIM

Complete investigation regarding the dairy products found on the free market and the hygiene quality evaluation with special emphasis on the pathogen bacteria prevalence;

MATERIALS:

This research has been carried out on a number of 120 samples of buffalo dairy products: milk, cheese, mozzarella and telemea.

❖ For comparative assessment, 30 samples of buffalo dairy products were taken from the hypermarkets.

PCR METHOD:

❖ FAST ID (Promega) extraction kit; DNA read with a UV-VIS spectrophotometer (Nanodrop, ND-1000, Thermo-Scientific, USA)

❖ Simplex and duplex PCR amplification method:

Specie	Primer sequence	Target Gene (product's size/bp)	Bibliographic reference
Cow	ACT AGA TCA CGA GCT TGA TCA CCA TGC **(F)** ATG CCT GGT AAA ATT CAT TAA ATA GCG **(R)**	DNAmt/ 126 bp	Sachinandan D et al. 2011
Buffalo	ACT AGA TCA CGA GCT TGA TCA CCA TGC **(F)**	DNAmt/ 226 bp	Sachinandan D et al. 2011
	ACT AGA TCA CGA GCT TGA TCA CCA TGC **(R)**		

THE MICROBIOLOGICAL EVALUATION:

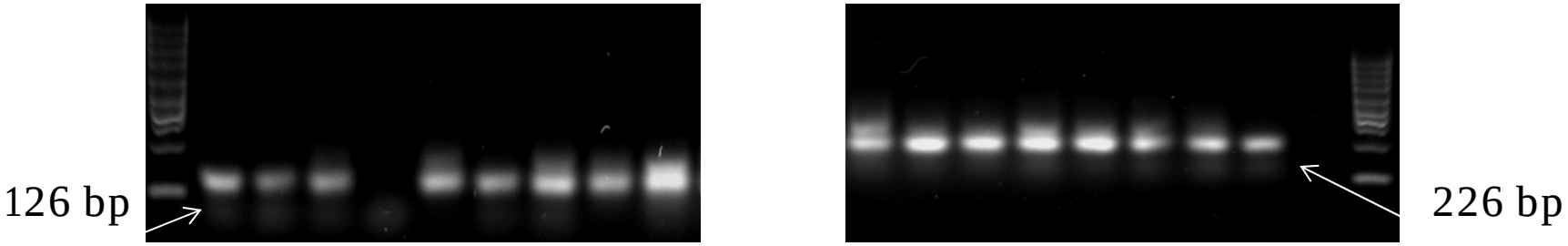
Classical methods: (NTGm.a) SR ISO 4833/2003; *Listeria spp.:* SR EN 11290/1/2000; *E.coli*: SR ISO 7251/1996; *Salmonella*:ISO EN 6579/2003; *Staphylococcus spp.* SR EN 6888/1/2002; *Bacillus cereus:* SR EN 7932/2003.

Confirmation methods: TREK system, VIDAS system, VITEK system and PCR.

Organsim	Secvența Primerilor/ Primer sequence	Gena ținta, (mărimea produsului/pb) Target Gene (product's size/bp)	Referință bibliografică/ Bibliographic reference
E.coli	**GAAGAGTCCTGGGATTACG (F)** **AGCGATGCAGCTATTAATAA (R)**	Toxina VT1 subunitatea B (130)	Pollard şi col. (1990)
Salmonella	**TGTTGTGGTTAATAACCGCA (F)**	16S ARNr (571)	Lin şi Tsen (1995)
	CACAAATCCATCTCTGGA (R)		
L. monocytogenes	**GACATTCAAGTTGTGAA (F)**	Listeriolizina O (560)	Thomas şi col. (1991)
Listeria primer set	**CGCCACACTTGAGATAT (R)**		

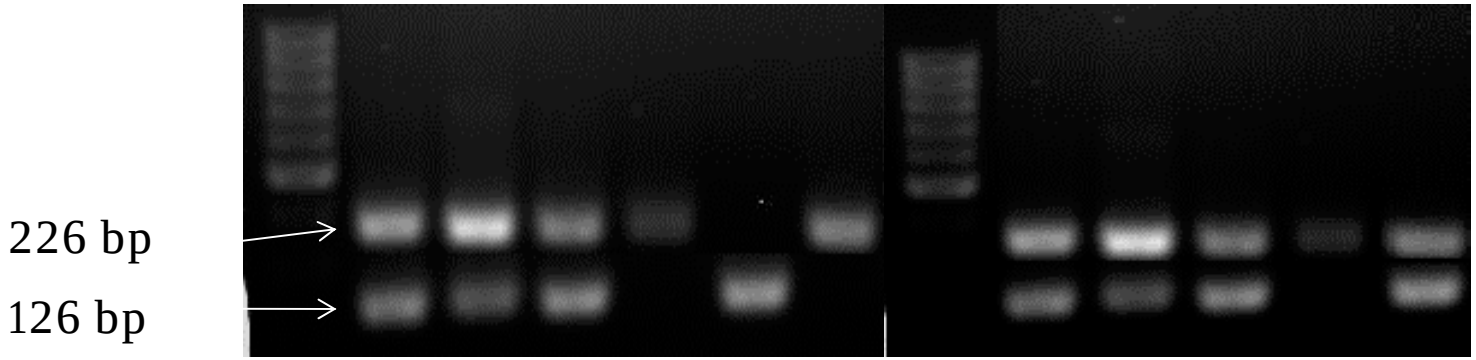
RESULTS:

❖ The majority of the samples (89%) collected from the traditional market were mixed with bovine milk.



The amplification results with cow/buffalo specific primers. The lines are: L – ladder 100 bp, $L_1 – L_9$ DNA Buffalo Telemea/ $L_1 – L_9$ DNA Buffalo Telemea; L – ladder(100 bp)

❖ The highest percentage of adulteration was found at cheese and telemea (87%) while the lowest percentage in raw milk (23.2%)

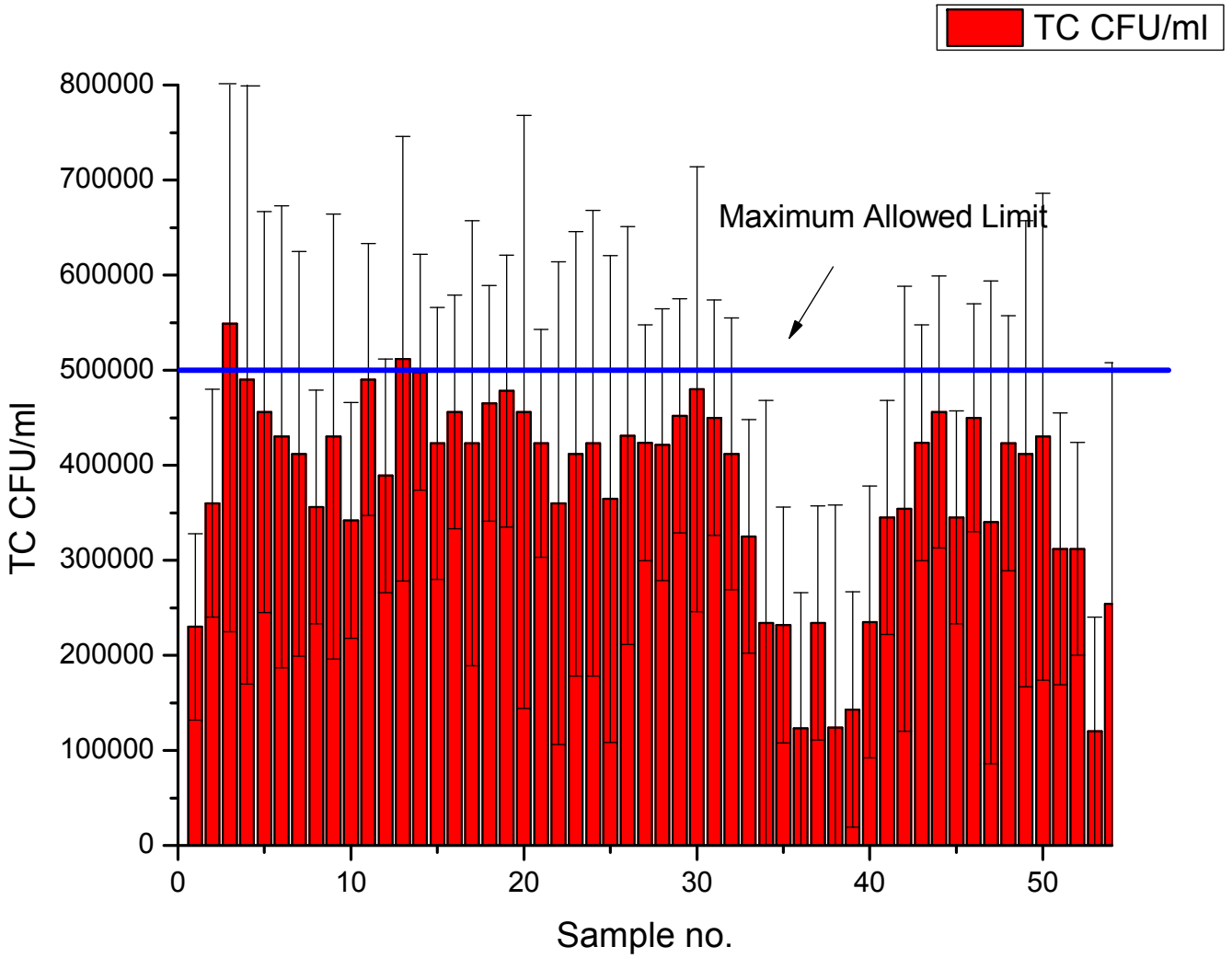


The amplification results with buffalo and cow specific primers. The lines are: L- ladder 100 bp, L_1 buffalo milk DNA; $L_2 – L_3$ DNA Buffalo Telemea; $L_4 – L_6$ Buffalo cheese; L_7 Positive Control/ L – ladder (100pb); $L_1 – L_3$ DNA Buffalo Mozarella; L_4 Negative Control; L_5 – Positive Control.

❖ The adulterations in high amount with cow milk were noticed also at the sensorial examination;

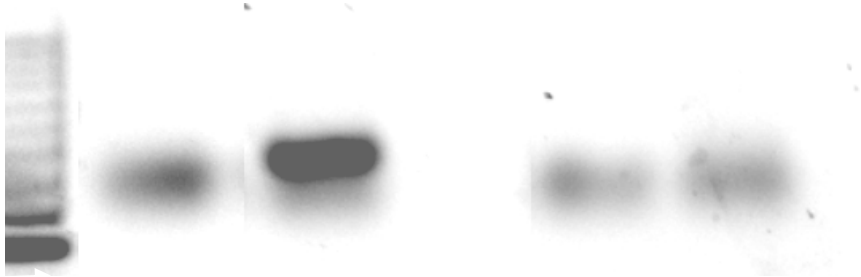
BUFFALO BUFFALO + COW

Total Plate Count (TC)

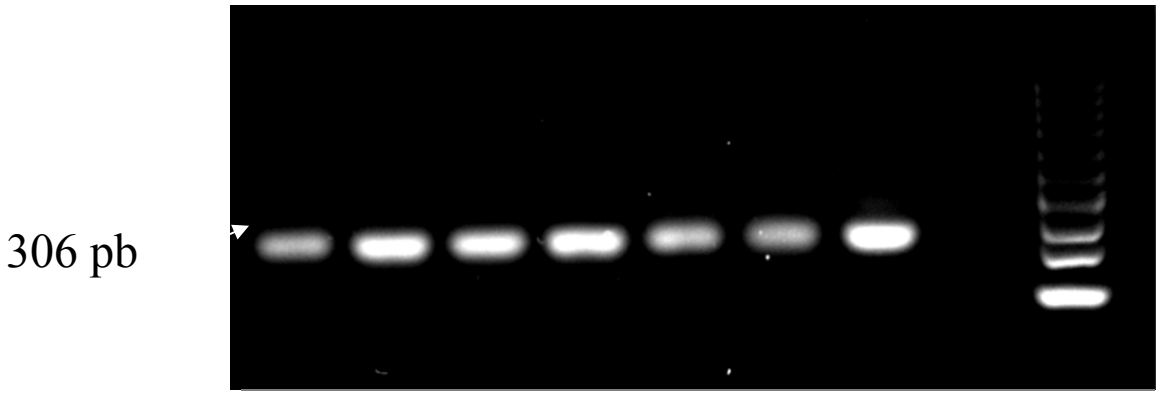


❖ Bacteria load evaluation

Microorganism	No. positive samples	%
Staphylococcus intermedius	2	5
Staphylococcus chromogenes	1	2.5
Staphylococcus caprae	1	2.5
Staphylococcus aureus	4	10
E.coli	34	86
Bacillus cereus	4	10
Listeria ivanovii	10	25
Listeria welshmeri	13	32.5
Listeria monocytogenes	0	0

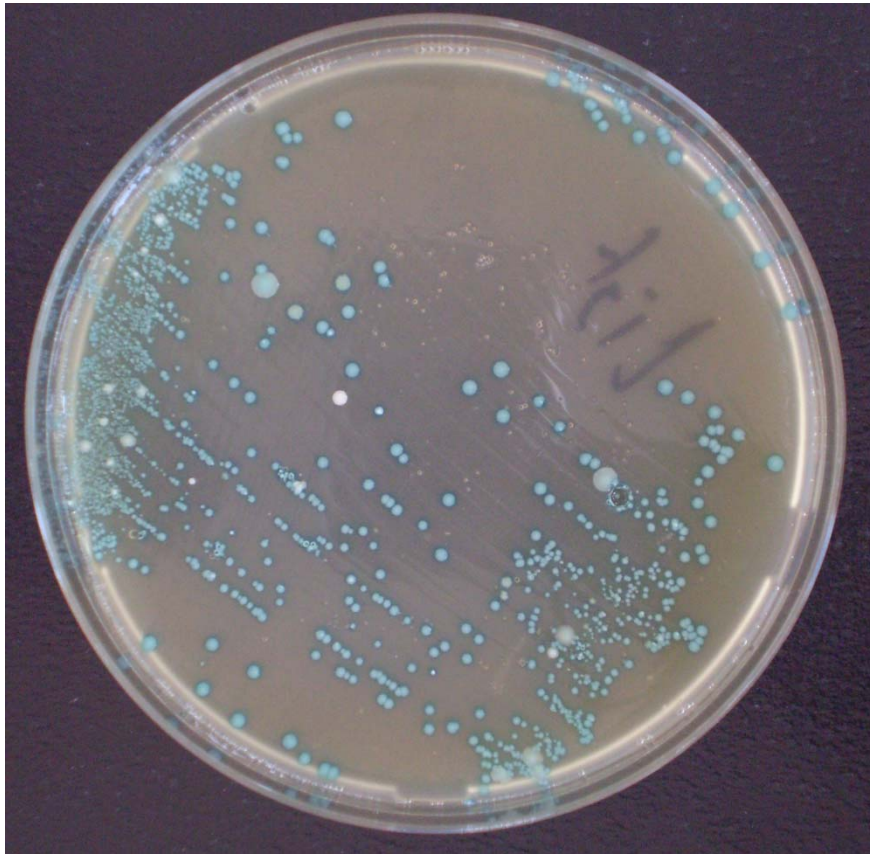


The electrophoretic profile of PCR positive products for Staphylococcus aureus isolated from raw buffalo milk



The electrophoretic profile of PCR positive products for Listeria spp. isolated from raw buffalo milk obtained in small scale producing units

Product	Microorganism	Free market		Hypermarket		ANOVA
		Positive samples	%	Positive samples	%	
Telemea	*Staphylococcus intermedius*	5	3	1	1	*
	E. coli	23	13	8	8	*
	Listeria welshmeri	2	1.2	-	-	ND
Fresh cheese	*E. coli*	21	26	11	14	*
	Listeria ivanovii	4	4.95	-	-	ND
	Stapylococcus spp.	10	12	2	2.5	*



❖ In the milk samples collected from the hypermarkets, none of the mentioned bacteria found on the traditional market could be detected;

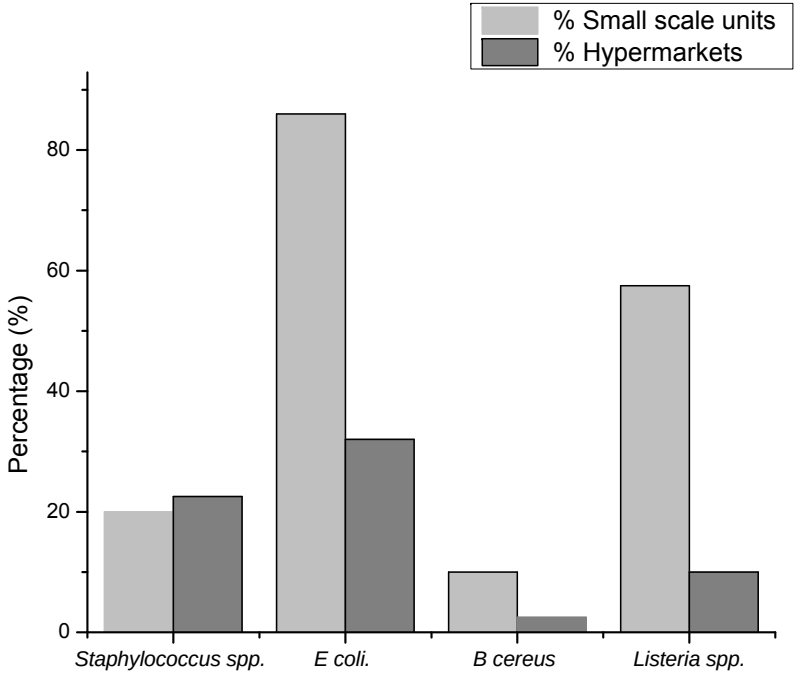
❖A very important cause is the fact that on the specialized markets, milk is sold only after proper processing (pasteurization) preventing the occurrence of microorganisms.

❖Of great concern is also the fact that in the traditional system, the pathogenic *Bacillus cereus* was found in four samples of raw milk;

❖ Most of the bacteria found in the dairy products collected from the free market are not pathogenic to humans;

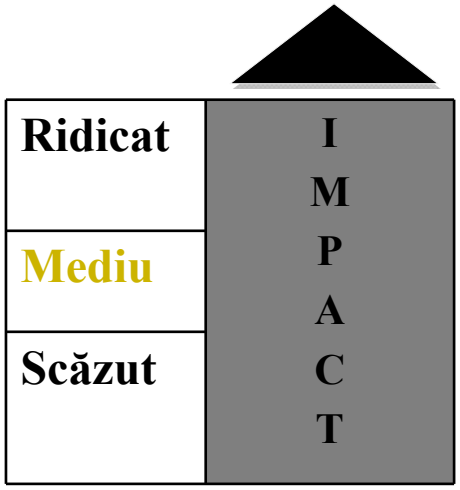
❖ Statistically, analyzing the obtained results significant differences ($p \leq 0,05$) among the number of bacteria isolated from products and raw milk were found, the highest level being found in the last mentioned.

❖ The *Listeria spp*. isolation frequency was significantly different ($p \leq 0,05$) at the raw milk samples collected from the traditional market than the one found at the industrial processors (hypermarkets).



RISK ANALYSIS

❖ Following the microbiological hazards' assessment at the dairy products obtained in traditional system it was established that the contamination probability with pathogens is average;
❖ The storage and processing steps favor the bacteria development in these type of products;
❖ The qualitative and quantitative evaluations on the risk impact were transposed into quality scales, which reflected the importance perceived in raport with the objectives.



In these small scale units, the growth of bacteria hazards was established to be due to:

- Non proper hygiene in the processing system;
- Manual milking without properly cleaning of the udder;
- The lack of knowledge of the workers for these microbiological hazards;
- The packing methods in unsterile recipients

Due to the fact that the HACCP program is not implemented, the monitoring, evaluation and prevention of risks are very low. Taking this fact into account, it is mandatory to periodically perform sanitary controls to guarantee product's safety and to eliminate possible public health contaminations.

CONCLUSIONS

The analysis of a range of commercial buffalo dairy products from various sources showed that adulterated buffalo products are present on the Romanian market and that appropriate monitoring is required to prevent consumer deception.

In addition, the achievement of hygiene in buffalo dairy small scale farms directly influences the production oriented economic results and health safety perspectives in human beings.

It is therefore critically important to ensure high quality buffalo raw milk production from healthy animals under good hygienic conditions and to apply control measures to protect human health.

We recommend that training and guidance should be given to farm' owners and their workers responsible for buffaloes' milking. Meanwhile, information on health hazards associated with contaminated or adulterated raw milk should be extended to the public, so that consumption of improperly processed dairy products could be avoided.

THANK YOU FOR YOUR ATTENTION!!!