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Microbial contamination and biochemical tests for Enterobacteriaceae in poultry eggs under conditions of intensive and extensive growth in the Korça region, Albania

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ABSTRACT— The eggs are widely used in the food industry and in consumer diets. The egg is well preserved in natural conditions because it has physico- chemical protectors. If they are cracked and damaged, this natural defense is lowered and the egg becomes a conducive environment for the growth of microorganisms, which endanger the health of consumers. Therefore, it is important that they are safe and microbiologically clean. Egg production (from chickens) in the Korça area is dominated by poultry, but also small producers (village). In this study, the general microflora of eggs on the surface (shell) and inside them was determined. Eggs obtained directly from the production sites were studied: Korça poultry and small farms of villagers stored at room temperature and some of them stored in the refrigerator (cooling). The number of microorganisms on the surface and inside in all samples (selection / incidence method) was compared. The study also consists in determining the pathogenic microflora for Enterobacteriaceae. Biochemical tests were performed to identify the presence of *Salmonella spp*. to eggs. Experimental measurements show: the total microbial load is higher than the surface of eggs inside them. The largest amount of microorganisms was counted on the surface of eggs taken from small farms than from Korça poultry plants. From the analyzed samples it resulted that, there was no presence of *Salmonella spp*.

KEYWORDS: food Safety, Poultry plant, farm poultry, general microflora, Salmonella spp.

1. INTRODUCTION

Fresh chicken eggs are one of the animal products with high nutritional value, widely used all over the world, for fresh consumption or for the processing and production of food products [1]. The quality and composition of fresh chicken eggs depends on the genetic material, the nutrition, the storage conditions of the eggs. Determining the quality and composition of eggs is done by evaluating and measuring a number of external and internal indicators. The Albanian consumer is becoming more and more aware of the impact that consumed food products have on his health [11]. Now, are selected quality food products and reasonably priced which evidences a real purchasing power of the consumer. Currently, Albanian companies launch poultry products (eggs) in competition with products of their sisters at home and abroad. It is this competition that requires a clear and complete system of control and certification [12]. The prospects of the country in terms of the use of this food require strict hygienic-sanitary and veterinary measures, optimal parameters of storage of products in refrigeration systems, implementation of existing laws, accurate certification of domestic products, implementation of new norms and rules, in line with those of the EU, to prevent and manage the potential risk of these products [10]. The methodology of experimental work offers a range of experiments of biochemical and microbiological nature of quality control as well as modern methods to reduce microbial contamination of these products, to prevent and stop the risk they offer through the biota of contamination [5].

This study aims to determine:

1. General microflora on the surface of eggs in the Agar-blood terrain under the conditions of storage of eggs at room temperature (natural conditions) and cooling (refrigerator)
2. Isolation of pathogenic microorganisms (Salmonella spp.; E. coli) on the surface of eggs to Korça poultry
3. Performing sequential and biochemical tests for accurate identification of colonies: Escherichia coli; Salmonella spp;
4. Preparation of simple preparations and coloring by gram for detailed identification of colonies

2. MATERIAL AND METHODS

The sampling method for analysis is: Method Incidence/selection Samples: The eggs in Korca Poultry for the purposes of this study, we performed microbiological analysis, for the determination of the general microflora on the eggs surface, of all types of samples. The terrains we used to do microbiological analysis are:
1-PCA terrain, for general micro flora; 2- blood agar terrain, for general microflora;3-Çapek terrain, for mold;4-DC terrain for pathogenic microflora General microflora on surface of eggs. We took some eggs at random way, in battery with sterile pincers, then in boxing switched alcohol lamp. We took material from the surface of eggs (shells) with sterile tampon and passed on PCA terrain, blood agar terrain and Capek terrain. It worked two parallel for each sample in number plates as the number of eggs taken for examination (for each terrain). The plates back overthrown and incubation in thermostat in 37^0C temperature. The plates were read after 24 hours, after 48 hours, after 7 days for mold [5], [6].

General microflora inside the eggs. For the egg microflora inside. We light the lamp with alcohol. We prepared in advance 15 erlenmeyer with sterilized water (autoclave tap water). In the case of performing microbiological analyzes inside the eggs, the method of successive dilutions was applied, respectively: 1:10 (first dilution); 1: 100 (second dilution); 1: 1000 (third dilution) [5].

Mode of action. Samples were taken uniformly (egg suspension 10 ml), under sterile conditions. The microbiological control was performed taking into account the control methods provided in practice for microbiological analysis. PCA terrain were used for bacteria, and Çapek for fungi, DC terrain was used for pathogenic microflora. The method of covering (planting) in petri plates was used. Two parallels were worked on for each sample. In the Petri plates where we have poured the suspension of 1 ml taken from each dilution made for each egg, the melted and cooled nutrient terrain is emptied up to 40-45 ^{0}C by rotating the plate in such a way that the suspension is homogeneously mixed with terrain. After solidification of the nutrient medium, the plates are turned upside down to avoid condensation dripping from the lid onto the terrain and placed in a thermostat at 37 ^{0}C to allow microorganisms to grow. Then the counting is done after 24 hours, after 48 hours and after 7 days for molds.

Pathogenic microflora on the surface and inside of eggs. DC terrain was used, to determine the pathogenic microflora, mainly for the identification of Salmonella spp. We used sterile tampons in test tubes to get the material on the surface of the eggs. We took the material with one hand and marked a number on each test tube. In boxing we passed the material obtained by lining on the DC terrain. [13] After reviving the sample in Selenium terrain we then spent it in DC field incubation time in thermostat is 24 and 48 hours [7]. We then performed biochemical analyzes for Enterobacteriaceae, where we passed colonies grown from Hayn Indol terrain blood Agar and Pepton Water. Here we identify if the colonies are: E. coli, Salmonella spp. or Staphylococcus spp. Incubate at 37^0C for 24 hours. After 24 hours we perform the test. We throw Indol with dots. If the ground turns red it is E. coli, if the ground does not turn red it is Staphylococcus aureus. Staphylococcus aureus has no gas formation while E. coli has gas formation [8].

3. RESULTS

The results for general microflora on the surface of eggs ”Korca Poultry” are given below:

Tables 1. The general microflora on surface of eggs in “Korca Poultry”

Surface of eggs (shells)	Parallels	Terrains						
		PCA		Agar Blood		Çapek		
		Incubation time		Incubation time		Incubation time		
		24 hours	48 hours	24 hours	48 hours	24 hours	48 hours	7 hours
Egg 1 CFU/egg	I	80	95	100	120	0	0	1
	II	70	80	115	125	0	1	1
	Average	75	87,5	107,5	122,5	0	0,5	1
Egg 2 CFU/Egg	I	75	80	90	100	0	0	2
	II	80	90	95	105	0	0	1
	Average	77,5	85	92,5	102,5	0	0	1.5
Egg 3 CFU/egg	I	90	95	106	114	0	1	2
	II	100	105	115	120	0	2	2
	Average	95	100	110,5	117	0	1,5	2
Egg 4 CFU/egg	I	84	90	98	105	0	2	2
	II	80	85	108	115	0	0	1
	Average	82	87,5	103	110	0	1	1,5
Egg 5 CFU/egg	I	100	110	115	125	0	1	1
	II	105	112	120	130	0	0	1
	Average	102,5	111	117,5	127,5	0	0,5	1

The number of colonies for each egg for all three types of terrains increases depending on the increase of the incubation time. But in all the analyzed samples the values of the total microbial microflora on the surface of the eggs are within the allowed norms (200 cfu).

The results obtained for the general bacterial microflora on the surface of the eggs, produced by chickens under conditions of extensive growth (from the farm poultry of the village) are reflected below (table 2):

Table 2. General microflora on the surface of eggs from farm poultry of the village

Surface of eggs	Parallels	Terrains						
		PCA		Agar blood		Capek		
		Time of incubation		Time of incubation		Time of incubation		
		24 hours	48 hours	24 hours	48 hours	24 hours	48 hours	7 days
Egg 1 CFU/egg	I	100	110	200	210	0	0	2
	II	108	120	198	204	0	1	2
	Aver.	104	115	199	207	0	0,5	2

Egg 2 CFU/egg	I	115	120	180	200	0	1	2
	II	120	130	190	205	0	1	3
	Aver.	117,5	125	185	202,5	0	1	2,5
Egg 3 CFU/egg	I	95	100	175	185	0	1	3
	II	100	115	170	190	0	2	3
	Aver.	97,5	107,5	172,5	187,5	0	1,5	3
Egg 4 CFU/egg	I	110	120	150	160	0	2	2
	II	120	135	155	165	0	2	4
	Aver.	115	127,5	152,5	162,5	0	2	3
Egg 5 CFU/egg	I	120	125	160	170	0	0	1
	II	130	135	165	180	0	1	3
	Aver.	125	130	162,5	175	0	0,5	2

General microflora inside the eggs

Regarding the results for the general bacterial microflora inside the eggs, produced by chickens under conditions of intensive growth (Korça poultry) they are all negative. For each egg and for both types of terrains used, in all three dilutions carried out we have no increase at all, despite the incubation time, which means that no colony was identified. So in all the samples analyzed the values of the total microbial microflora inside the eggs are zero. This can be explained by the presence of the egg cuticle [2] The results for the general bacterial microflora inside the eggs, produced by chickens under conditions of extensive growth (from the farms of the village) are reflected below (table 3):

Table 3. General microflora inside the eggs in three dilutions, for eggs from the village poultry farm

Samples	Dilutions	Parallels	Terrains				
			PCA		Capek		
			Time of incubation		Time of incubation		
			24 hours	48 hours	24 hours	48 hours	7 days
Egg 1 CFU/egg	The first dilution	I	0	0	0	0	0
		II	0	0	0	0	0
		Aver.	0	0	0	0	0
	The second dilution	I	0	0	0	0	0
		II	0	0	0	0	0
		Aver.	0	0	0	0	0
	The third dilution	I	0	0	0	0	0
		II	0	0	0	0	0
		Aver.	0	0	0	0	0
	The first dilution	I	84	90	0	2	3
		II	80	94	0	1	2
			82	92	0	1,5	2,5

		Aver.					
Egg 2 CFU/egg	The second dilution	I II Aver.	70 65 67,5	74 70 72	0 0 0	1 1 1	2 1 1,5
	The third dilution	I II Aver.	55 51 53	60 66 63	0 0 0	1 0 0,5	1 1 1
Egg 3 CFU/egg	The first dilution	I II Aver.	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
	The second dilution	I II Aver.	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
	The third dilution	I II Aver.	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
	The first dilution	I II Aver.	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
Egg 4 CFU/egg	The second dilution	I II Aver.	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
	The third dilution	I II Aver.	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
Egg 5 CFU/egg	The first dilution	I II Aver.	80 70 75	84 75 79,5	0 0 0	2 2 2	3 2 2,5
	The second dilution	I II Aver.	58 50 54	60 55 57,5	0 0 0	1 1 1	2 1 1,5
	The third	I II	50 44 47	58 50 54	0 0 0	1 0 0,5	1 0 0,5

	dilution	Aver.					

Of all the samples analyzed only in two of them was microbial growth identified. The number of colonies for these eggs for both types of terrains used, increases depending on the increase of the incubation time, and decreases passing from the smallest to the largest dilution. The existence of these two such samples indicates that these eggs have damaged their cuticle, or their shell may have suffered cracks or fissures [1].

Eggs kept in refrigerated conditions, of both types of samples, eggs from Korça poultry and eggs from farms in the village, had similar results. In about 60% of them, no microbial growth on the surface of the eggs was identified at all for the three types of terrains used, that of PCA, Blood Agar, and Capek. Only in about 40% of the samples a small number of colonies were identified, and this in the Capek terrain, and only on the surface of the eggs taken from the farmers of the village (even those kept in refrigerated conditions). As for the inside of the egg, for all the analyzed samples, and for both their types (eggs from Korça poultry, eggs from the farms village) no colony was identified. As for the inside of the egg, for all the analyzed samples, and for both their types (eggs from Korça poultry, eggs from the farms of village) no colony was identified.

Table 4. Number of E.coli and Salmonella spp. colonies on the egg surface, eggs from Korça poultry (in DC terrain)

Samples	*E.coli* (cfu)	*Salmonella spp.* (cfu)
Egg 1	2	0
Egg 2	0	0
Egg 3	1	0
Egg 4	3	0
Egg 5	1	0

Table 5. Number of E.coli and Salmonella spp. on the egg surface, eggs from the village (DC terrain)

Samples	*E.coli* (cfu)	*Salmonella spp.*(cfu)
Egg 1	4	0
Egg 2	1	0
Egg 3	2	0
Egg 4	5	0
Egg 5	1	0

Biochemical tests for the identification of pathogenic microorganisms were performed on the egg surfaces of both types of samples. Hayn Indol and peptone water after 24 hours of incubation, when we added the Indoli drops turned red. This indicates the presence of E.coli. E.coli is a sanitary index. E.coli indicates the level of hygiene of the place where the chicken egg was released etc...

Table 6. Biochemical tests for the identification of pathogenic microorganisms (with indole in Hayn Indol media and pepton water) for the egg surface

Samples	**Samples**	**Hayn**	**pepton water**	**Indol**
Veza 1	Eggs from Korca poultry	+	+	Red color
Veza 2		+	+	Red
Veza 3		+	+	Red
Veza 4		+	+	Red
Veza 5		+	+	Red

Veza 6	Eggs from farm poultry	+	+	Red
Veza 7		+	+	Red
Veza 8		+	+	Red
Veza 9		+	+	Red
Veza 10		+	+	Red

From the (tables 4,5 and 6) above it is clear that the number of E. coli colonies is greater in the eggs obtained from the farms of the village than in the eggs obtained from the Korça poultry. E. coli is the normal flora of the anal part as a microorganism of fecal contamination. Number of Salmonella spp. in these eggs it was zero. This is a very good indicator of the quality of poultry eggs.

4. DISCUSSIONS

At the end of this study on the general microbial contamination of eggs produced by chickens in conditions of intensive and extensive growth in the city of Korca we reached the following conclusions:

From the results obtained from microbiological analyzes for the general microflora on the surface and inside of eggs in PCA, Blood Agar and Çapek terrains we can say that:
Number of colonies on the surface of eggs obtained from Korça poultry:

- in the terrain PCA varies from 75 to 102 cfu (average number) after an incubation time of 24 hours, and from 85 to 111 (average number) after an incubation time of 48 hours
- In the terrain Agar blood varies from 152 to 199 cfu (average number) after an incubation time of 24 hours, and from 162 to 207 (average number) after an incubation time of 48 hours
- in Çapek terrain (for molds) this number varies from 0.5 to 1.5 cfu (average number) after an incubation time of 48 hours, and from 1 to 2 cfu (average number) after an incubation time of 7 days

Number of colonies on the surface of eggs taken from farms in the village to Korca country:

- in the terrain PCA varies from 97 to 125 cfu (average number) after an incubation time of 24 hours, and from 107 to 130 cfu (average number) after an incubation time of 48 hours
- In the terrain Agar blood varies from 152 to 199 cfu (average number) after an incubation time of 24 hours, and from 162 to 207 cfu (average number) after an incubation time of 48 hours
- in Çapek terrain (for mold) this number varies from 0.5 to 2 cfu (average number) after an incubation time of 48 hours, and from 2 to 3 (average number) after an incubation time of 7 days

Regarding the interior of the eggs taken from Korça poultry, we have no increase at all, so no colony was identified. This shows that these eggs are of a very good quality, and also the production conditions of these eggs which directly affect the quality must be within the norms (up to 200 cfu).
Analyzes for the inside of the eggs taken from the village showed that only in two of them we had microbial contamination. The number of colonies (average number) in these eggs varies:

- PCA terrain according to all three dilutions; in the first dilution from 75 to 82 cfu, in the second dilution from 54 to 67 cfu, in the third dilution from 47 to 53 cfu, after an incubation time of 24 hours. Whereas after an incubation period of 48 hours the number of colonies varies; in the first dilution from 79 to 92 cfu, in the second dilution from 58 to 72 cfu, in the third dilution from 54 to 63 cfu
- in Çapek terrain (for molds) according to all three dilutions; in the first dilution from 1.5 to 2 cfu, in the second dilution 1 cfu, in the third dilution 0.5 cfu, this after an incubation time of 24 hours. Whereas after an incubation period of 48 hours the number of colonies varies; in the first dilution 2.5 cfu, in the second dilution from 1.5 cfu, in the third dilution from 0.5 to 1 cfu

Eggs kept in refrigerated conditions, of both types of samples. In about 60% of them no microbial growth was identified on the surface of the eggs. Only in about 40% of the samples a small number of colonies were identified, and this in the Çapek terrain, and only on the surface of the eggs taken from the farmers poultry of

village. As for the inside of the egg, no colony was identified
Eggs produced by chickens under conditions of intensive growth (eggs from the farm poultry in village to Korca) have a significantly higher number of colonies of bacterial microflora in their shell than eggs produced by chickens under conditions of intensive growth (Korça poultry)
Colors of bright red color were observed in DC terrain. These colonies are E. coli. The presence of E. coli microorganisms (in farm poultry), may also have come as a result of poor hygiene of the place where the chickens laid their eggs.

In none of the samples, both in Korça poultry eggs and in those taken from farm poultry in the village was the presence of any colony of Salmonella spp. This indicates that these eggs are not contaminated and are safe to be used as food by humans.

5. ACKNOWLEDGEMENT

This research study on microbiological safety of eggs in some poultry to Korca country, was performed in poultry premises. The microbiological analyzes of this study were determined in the microbiology laboratory of the sanitary center in the city of Korca. The experimental work presented in this study research is only a small part of the scientific research and evidence that can be done, on the results of which depends the successful continuation of research in the same field.

6. CONCLUSIONS

At the end of this study we are giving some (modest) valid recommendations: Relevant institutions which are responsible for the safety of food products, as well as eggs, found in the market should carry out more frequent checks of the safety elements that must meet these products. All those who produce and sell eggs in the market must be inspected and ensure a safe product without microbial contamination. To prevent bacteria in food and eggs it is also recommended that they be stored at a low temperature, below 4ºC minimum, no bacterial growth occurs here. Important factors to reduce bacterial contamination on and in the eggs are: proper cooling, and humidity conditions, proper washing and that the eggs are treated more carefully to avoid breaking them. It is also important that, if possible, eggs are stored for a short time in an environment where cross-contamination occurs easily, such as stables. Consumers should always wash the eggs before using them for cooking, they should also make sure that the origin of the product of their choice is known and safe.

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