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I. – Introduction

Eggs contaminated with micro-organisms play a significant role in poultry production pathology and in the spreading of diseases. Micro-organisms cause increased mortality of embryos, lower hatchability and increased early chick mortality. Infections of humans are also common.

Ardrey et al. (1968) showed that layers with pathogenic Escherichia coli isolated from their droppings laid contaminated eggs at the level of 2.7%. They also supposed that layers with E. coli in their oviduct transmitted through their eggs the same bacteria counts to chicks which were underweighted after hatching very often with retention and infection of vitellus. In his study, Gross (1984) reported that bacteriologically "clean" eggs under good incubation conditions diminish the transmission of E. coli embryos, their mortality and also the inflammation of omphalitis and early chick mortality.

In healthy flocks in the dropping of which no salmonellas were found, Dorn et al. (1984) isolated unspecific salmonellas in 0.13% of the shells of table eggs which were microscopically clean and in 0.04% of the eggs soiled by droppings while in the yolks no salmonellas were found. In diseased flocks (tests on salmonellas in the droppings were positive) eggs shell salmonellas were isolated at the level of 0.056% on the floor and 0.086% in cages. The difference is not statistically significant. The suggest that salmonellas can penetrate the egg shell regardless of hen contamination and, though little in number (0.081% on average), they can be dangerous for humans.

Along with Escherichia coli and salmonellas there is also Compylabacter jejuni which can provoke infections in humans. It was isolated in healthy poultry at the level of 80%. Neill et al. (1985) and Clark and Bueschkems (1985) contaminated eggs artificially with Compylobacter jejuni but they did not prove the transmission of bacteria to chicks. They proved that bacteria died six hours after penetrating the egg shell and entering the egg.

Smeltzer et al. (1979) examined the level of contamination by micro-organisms. The penetration of egg shells and egg contents has been shown in table eggs at the level of 9.2% and in hatching eggs at the level of 16.5% in the floor position of hens. They found that the main reason for egg contamination with various micro-organisms was wet and soiled litter. Spark and Board (1985) showed that the moisture content in newly laid eggs diminishes the ability of cuticle to protect the egg contents. With so-called bedwet eggs, drops of water penetrate the cuticle, change its structure and enable micro-organisms to enter the egg contents immediately after laying. Authors think that the infection of eggs is possible at the time of laying and also after washing. According to Holley (1986), eggs may be washed few hours after laying. When eggs (the shells of which were artificially contaminated with S. typhimurium were washed with cold water at pH from 10.5 to 11.0, salmonellas were destroyed in five hours, but when the pH was lower than 9.5 this did not work. The author concludes that pH is one of the most important factors for the elimination of salmonellas from egg shells. To isolate the salmonella counts in table eggs, Hafez et al. recommended a simple method to demonstrate their presence. The eggs from contaminated breedings have to be incubated at 37°C for seven days and then bacteriologically treated to selective growing media and the isolation of gramnegative bacteria.

Bacteria strains resistant to antibiotics can also be transmitted by contaminated eggs. Bećirević (1983) examined the shells of 660 eggs and isolated resistant salmonella strains at the level of 70%, Proteus strains at the level of 72%, E. coli at the level of 77% and Pseudo-manas aeruginosa at the level of 100%. She has shown that the most resistant strains transmit the resistance to the recipients.

II. – Materials and methods

With regard to significant early chick mortality, we made several bacteriological tests on eggs, believing that contaminated eggs could cause bacterial diseases of chicks. We intended to investigate the level of shell contamination with bacteria and molds to prove or disprove successfulness of sanitary treatment of eggs on farms and hatcheries ; we also investigated the presence of bacteria in the yolk and their efficiency in spreading disease.

In our investigations we examined table eggs from one farm and hatching eggs from four farms. To determine the presence of bacteria and molds on the egg shells, we used the method of dump swabs of the exposed parts of the shell (30 eggs) placed in filler flats. When the samples of 30 eggs contained more than one million colonies of bacteria, the shells were considered to be hygienically unacceptable. To isolate bacteria in the yolk we bacteriologically examined each egg which had been washed previously in alcohol under clean conditions. We used neutral agar, Sabouraud's agar, Selenit bujon and brilliant green agar to isolate micro-organisms ; their identification was by the usual laboratory method, according to Bargey (Kreig and Molt, 1984).

III. – Results

The results of these investigations are shown in six tables. **Tables 1**, **2** and **3** show the level of contamination of the hatching egg shells with micro-organisms, before the sanitary treatment by gaseous formaldehyde on farms and hatcheries.

The results in **Table 1** show that, before sanitary treatment, shells were hygienically unacceptable at the level of 71.68% on average on farms and hatcheries. The reason is damp and soiled litter, irregular collecting of eggs, as well as unhygienic transport and storage of eggs. The effects of gaseous formaldehyde on bacteria and molds from hatching egg shells on farms and hatcheries are shown in **Table 2.**

From the results shown in **Table 2** it is obvious that shells of sanitary treated eggs on farms and hatcheries are contaminated with bacteria and molds at the level of 27.2% on average. According to Kralj *et al.* (1986) the consequences are lower hatchability (from 80% to 82% only), increased number of omphalitis and yolk sac inflammation (about 10%) and increased chick mortality in the first two weeks of production (about 8% on average).

These results show that salmonellas were isolated in 1.08% of hatching eggs and in 0.48% of table eggs. After the hatching eggs were treated with gaseous formaldehyde, salmonellas were found in 0.009% of examined eggs.

The bacteria presence in yolks of hatching and table eggs in clinically healthy and contaminated breedings is show in **Tables 4** and **5.**

The results in **Table 4** show that table eggs of healthy breedings are contaminated with bacteria at the level of 0.40% only, and hatching eggs at the level of 10.10% of cases.

According to the results shown in **Table 5** we isolated salmonellas in eggs at the level of 1.50% to 5.86% of cases. In the cases of infection caused by Staphylococcus, we isolated Staphylococcus aureus in 40.91% of hatching eggs and in 1.67% of table eggs. The infection caused by *Pseudomonas aeruginosa* in heavy layer breedings was registered in only one case, and bacteria was isolated in 25.83% of cases.

The information on the level of shell and yolk contamination with bacteria in healthy commercial layers is very interesting. It is given in **Table 6**.

These results show that bacteria were isolated from the eggs shells at the level of 8.84% and from the yolks at the level of 0.36% of cases. Bacteria strains were : Escherichia, Bacillus and Actinomyces.

IV. – Discussion

Analysis of these results shows that the shells of sanitary untreated eggs are contaminated with bacteria and molds at the level of 71.68% of cases. After being treated with formalyn, eggs were still hygienically unacceptable at the level of 27.25% of cases. The most often present bactery strains were Escherichia, Salmonella, Proteus, Staphylococcus, Streptococcus, Bacillus, Micrococcus and Antinomyces and the most often mold strains were : Aspergillus, Penicillium, Fusarium, Rhizopus, Mucor and Cladosporium along with various yeasts.

Salmonellas were found on hatching egg shells at the level of 1.08% of cases and on table egg shells at the level of 0.48% of cases. Salmonella counts were diminished under the treatment with gaseous formaldehyde, but they were not destroyed, only lowered to 0.009%.

The consequences of such a situation are : lower hatchability (from 80% to 82%), increase of omphalitis and yolk sac inflammation (about 10%) and chick mortality in the first two weeks of production (about 8% on the average).

The percentage of hatching egg (yolk) contamination with bacteria is 10.10% and table eggs 0.40% and it is a significant difference. In diseased breedings the number of contaminated eggs is much higher than in healthy breedings and it is rather different with each infection. We found the hatching layers on a farm in SR Slovenia. Examining 222 egg contents, we isolated *S. enteritidis* in 5.86% of cases. In SR Croatia, on a farm of commercial layers, infection with *S. typhimurium* also appeared. It was isolated in 45 yolks of examined eggs in one or 2.22% of cases. On the same farm, *Salmonella virchow* was isolated in 2.22% of cases and *Salmonella agona* in 5.00% of cases.

On a second farm in the same republic, a chronic skin inflammation appeared, with characteristic purulent subcutaneous abscesses. By examination of abscess contents, we isolated *Staphylococcus aureus* and *C. perfingens* and in yolks of 22 eggs of diseased layers we isolated *Staphylococcus aureus* in nine or 40.9% of cases.

Pseudomonas aeruginosa was found on the farm of light strain layers in SR Crotia. We supposed that the layers were infested with fowl typhoid, but we could not prove specific salmonellas excepted infection with *Psuedomonas aeruginosa*. The bacteria was isolated in 93 eggs or 25.83% of 360 examined eggs.

We also searched the egg shells and yolks contaminated with bacteria on one farm of light layers in SR Croatia. The results showed that the egg shells and egg contents of table eggs were significantly less contaminated with bacteria than those of hatching eggs.

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Table 1: Findings of micro-organisms on sanitary untreated hatching egg shells on farms and hatcheries

Production	Number of eggs	Hygienically unacceptable	
		Number	%
Farms	1,285	925	71.98
Hatcheries	4,435	3,175	71.59
TOTAL	5,720	4,100	71.68

Table 2: Bacteria and molds findings on shells of sanitary treated hatching eggs

Place	Eggs in total	Hygienically unacceptable	
		Number	%
Farms	7,048	2,310	32.77
Hatcheries	9,422	2,181	23.15
TOTAL	16,470	4,491	27.27

Table 3: Salmonella findings on the egg shells

Treatment of eggs	Number of eggs	Salmonellas isolated	
		Number	%
	Hatching eggs		
Untreated eggs	650	7	1.08
Treated eggs	22,575	2	0.009
	Table eggs		
	630	3	0.48

Table 4: Bacteria findings on egg yolks of healthy breedings

Bacteria strains	Hatching eggs (n = 1911)		Table eggs (n = 742)	
	Number	%	Number	%
Escherichia coli	157	8.22	1	0.13
Coliform bacteria	17	0.88	-	-
Proteus vulgaris	8	0.42	-	-
Staphylococcus	6	0.34	-	-
Streptococcus	5	0.26	1	0.13
Bacillus cereus	-	-	1	0.13
TOTAL	193	10.10	3	0.40

Table 5: Bacteria findings on egg yolks of diseased breedings

N°	Bacterial infections	Bacteria strains	Hatching eggs			Table eggs		
			Number	Positive		Number	Positive	
				N°	%		N°	%
I	Salmonellosis	S. ententidis	222	13	5.86	200	3	1.50
II	Salmonellosis	S. typhimurium	30	1	3.33	45	1	2.22
III	Salmonellosis	S. virchow	-	-	-	90	2	2.22
IV	Salmonellosis	S. agnoa	-	-	-	60	3	5.00
V	Staphylococcosis	Staphylococcus aureus	22	9	40.91	60	1	1.67
VI	Pseudomoniasis	Pseudomonas aeruginosa	360	93	25.83	-	-	-

Table 6: Bacteria findings on table egg shells and yolks

Egg number	Shells		Yolks	
	Number	%	Number	%
1 390	123	8.84	5	0.36