

EVALUATION OF THE POST-CLOSURE CONTAMINATION DEGREE IN THE BROILER OF HERBAL

Elena Hriscu (Ursu)^{1*}, Irina Elena Ismană (Ciobotaru)¹, M.G. Usturoi²

¹Veterinary Safety Laboratory and Food Safety Iași, Romania

²University of Agricultural Sciences and Veterinary Medicine-Iasi, Romania

Abstract

The study was conducted on two growing farms, from which 80 samples (one day old chicks) were taken, half in the hot season and half in the cold season; practically, 40 samples were collected per season (2 popular series x 40 samples / season, ie 80 samples in the two seasons of two different farms).

Parameters analyzed were: *Salmonella* spp., *Escherichia coli*, *Staphylococcus* spp., *Streptococcus* spp. Using the national, standardized, validated and accredited RENAR working methods. Detection and isolation of bacterial strains was done on liquid and solid culture media for pre-enrichment, enrichment, isolation and biochemical identification. For confirmation, mini-API galleries specific to each category of bacteria were also used: *Salmonella* spp. and *Escherichia coli* ID 32 E; for *Staphylococcus* spp - galleries ID 32 STAPH and for *Streptococcus* spp - rapid ID 32 STREP.

From 40 samples analyzed in the summer season, the two growing farms resulted in 85% contaminated samples.

From the winter season, we examined 40 samples from the same growing farms and resulted in 10% of the contaminated samples.

From the analyzes performed, it emerged that day-old chicks were contaminated with the same types of bacterial strains, *Escherichia coli* and *Staphylococcus* spp., From both growing farms.

The highest incidence was with the *Escherichia coli* strain, at a rate of 70%; And the strains of *Staphylococcus* spp were found to be 25%.

Key words: isolation, identification, bacterial strains, day-old chicks

INTRODUCTION

The chicken is a remarkable achievement of genetics and nutrition, a realization that could be achieved due to scientific and technological advances [6].

After hatching the chickens are transported to the growing farm, in boxes and special machines to ensure the necessary transport conditions and they are disinfected before each transport.

Prior to popular halls, they are properly prepared to avoid contamination with various infectious agents that can cause illness and implicitly considerable mortality [1].

Even if the biosecurity conditions are strictly observed during the transportation

and the halls, there are still bacteriological mortality.

Prevention of diseases in poultry farms requires a complex program of biosecurity, vaccination and especially hygiene [3,4].

Disease prevention is based on a hierarchy of prophylactic measures such as hygiene and decontamination, vaccination, maintenance, nutrition and medication [5].

Most embryonic diseases are due to vertical transmission, and the following diseases can be transmitted in this way: salmonellosis, respiratory mycoplasmosis, avian pseudomonosis, colibacillosis, Newcastle disease, aspergillosis [2].

Due to these diseases, which can also occur in broiler chickens, we carried out bacteriological analyzes from the day-old chickens from the popular group of the two farms.

*Corresponding author:

dr_ursu_elena@yahoo.com

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From this point of view, we have tried to identify the contamination of day-old chicks from two different seasons, from summer to winter, from different breeding units.

Samples were tested from a bacteriological point of view using standardized and accredited RENAR working methods.

Samples were harvested from two different breeding farms of broiler chickens.

MATERIAL AND METHOD

The study was conducted on a number of 80 samples from two different growth farms by 40 samples / season. The period was divided into two seasons: one summer (June-August 2016) and one winter (December 2016-February 2017).

From each season, 20 samples of one day old chickens were harvested from the two farms, according to popularity.

The biological material consisted of one-day-old chickens after the two broiler breeding farms were populated.

These samples come from different breeding units.

To isolate and identify the microorganisms that can contaminate the samples, we performed the following steps:

1. Primary isolation stage - consists in stimulating the growth of bacterial strains on non-selective usual agar and nutrient broth.

After sowing the two media we thermostated at 37 ° C for 24 hours.

2. Identification stage - consists of the bird from the two media of the previous stage, on selective isolation media, depending on the characteristics of bacterial cultures development.

3. Biochemical identification and confirmation by miniAPI tests.

For the isolation and identification of *Salmonella* spp., We seeded from the liquid medium - nutrient broth, on selective AgarXLD (xylose-lysine-deoxycholate) agar medium. I thermostated at 37°C for 24 hours. After this period I read the plates, and we did not find microbial development specific to the development of bacteria of the genus *Salmonella* spp.

For the isolation and identification of bacteria of the genus *Escherichia coli*, we seeded from usual broth and nutrient agar

mediums on special diagnostic environments for the Enterobacteriaceae group, finding that in the nutrient broth we noticed intense turbidity, a ring on the surface and the nutrient agar were developed colonies of variable dimensions (2-6 mm diameter), opaque, unpigmented, type S. Crops smell of ammonia.

The special environments for this type of bacteria are XLD (xylose-lysine-deoxycholate agar), which have developed yellow colonies.

For the biochemical confirmation, the characteristics of these bacteria are: fermenting lactose and glucose, producing lysine decarboxylase, is indole positive, does not produce hydrogen sulfide, and does not use ammonium citrate as the sole source of carbon. For a secure confirmation we used the miniAPI ID 32 E galleries.

For the isolation and identification of bacteria of the genus *Staphylococcus* spp we seeded from the usual broth and nutrient agar medium. On the nutrient broth, staphylococci grow abundantly, with uniform turbidity and homogeneous storage, while the medium becomes clear. Frequently, on the surface forms an annular film. On the surface of the solid agar-nutrient agar, staphylococci form within 24 hours, under aerobic conditions, colonies with a diameter of up to 3 mm, and within 4-5 days their size is 3 to 10 mm. Datorită acestor caracteristici de dezvoltare a coloniilor am pasat pe mediul special de izolare Baird-Parker cu RPF.

On this selective isolation medium or developed black-gray colonies, surrounded by an opaque halo.

Bio API ID 32 STAPH galleries are used for biochemical confirmation.

To isolate and identify bacteria of the genus *Streptococcus* spp., The nutrient broth passes through the selective Edward agar isolating medium.

The appearance of colonies on this medium is small, fine, gray, metallic, but we have not found microbial growth.

RESULTS AND DISCUSSIONS

According to the legislation in force, the parameters analyzed are the following:

Salmonella spp., *Escherichia coli*, *Staphylococcus* spp. and *Streptococcus* spp.

Following these analyzes, we found that in both units the contamination of day-old chicks was with *E. coli* and *Staphylococcus* (fig. 1, 2).



Fig. 1 Typical colonies of *E. coli* on XLD agar



Fig. 2 Baird Parker medium - typical colonies of *Staphylococcus* spp

Due to the development of typical colonies on isolation environments, we also performed miniAPI galleries (fig. 3, 4).

The biochemical characteristics of *E. coli* bacteria are: They ferment lactose and glucose, produce lysine-decarboxylase, is indole-positive, does not produce hydrogen sulphide and does not use ammonium citrate as the sole source of carbon (fig. 3).



Fig. 3 Identification of *Escherichia coli* using API galleries ID 32 E

The biochemical characteristics of bacteria in the *Staphylococcus* genus are: they are positive for lactose, glucose, trehalose and mannitol; exhibits evident catalase activity, which allows the differentiation of streptococci, which are negative catalases (fig. 4).



Fig. 4 Identification of *Staphylococcus* spp using API galleries ID 32 STAPH

Of the total of 80 day old chicks, analyzed during the two seasons, of the two breeding farms, we obtained the following results:

”UNITATEA A”

Of the 20 samples analyzed in the summer season at a popular farm number in this unit we obtained 65% negative samples and 35% were positive, of which 25% positive for *E. coli* and 10% positive for *Staphylococcus* spp (tab. 1).

Table 1 Results in day-old chicks in unit "A"

The harvest period	Nr. analyzed samples	Nr. negative samples				Nr. positive samples			
		Salmonella spp	E.coli	Staphylococcus spp	Streptococcus spp.	Salmonella spp	E. coli	Staphylococcus spp	Streptococcus spp.
Summer 2015	20	20	15	18	20	-	5	2	-
Winter 2015	20	20	20	20	20	-	-	-	-

In the winter season out of the total of 20 samples analyzed all were negative.

”UNITATEA B”

A 50% of the 20 samples analyzed in the summer season were negative and 50% were positive, of which 35% were positive for *E.*

coli and 15% positive for *Staphylococcus* spp (tab. 2).

In the winter season of the 20 samples analyzed, 90% were negative and 10% of the samples were positive for *Escherichia coli*.

Table 2 Results in day-old chicks in unit „B”

The harvest period	Nr. analyzed samples	Nr. negative samples				Nr. positive samples			
		<i>Salmonella</i> spp	<i>E.coli</i>	<i>Staphylococcus</i> spp	<i>Streptococcus</i> spp.	<i>Salmonella</i> spp	<i>E. coli</i>	<i>Staphylococcus</i> spp	<i>Streptococcus</i> spp.
Summer 2015	20	20	13	17	20	-	7	3	-
Winter 2015	20	20	18	20	20	-	2	-	-

CONCLUSIONS

From the analyzes we performed, we found that day-old chicks were contaminated with the same type of bacterial strains, although the samples came from two different growth farms.

The highest incidence was with *E. coli*, which is also an indicator of hygiene, favoring development in a higher percentage, followed by bacteria of the genus *Staphylococcus* spp., But with a lower percentage.

The results show that in the summer season, the contamination of day-old chicks was higher than in the winter season; One reason could be the temperature difference between the two seasons, knowing that high temperatures favor multiplication of microorganisms.

RECOMMANDATION

Prevention of diseases in poultry farms requires compliance with a comprehensive program of biosecurity, vaccination and hygiene.

In the broiler chickens, the "empty, everything full" principle must be respected and the interval between depopulation and restocking should be at least 10 days.

Disease prevention is based on a hierarchy of prophylactic measures such as hygiene and decontamination, vaccination, maintenance, nutrition and medication.

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