RESULTS ON THE ASSESSMENT OF THE DEGREE MICROBIAL CONTAMINATION OF HATCHING EGGS

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Abstract

The study was conducted in two hatcheries, where many were taken 160 samples (embryonated eggs are in the 18th day of incubation), half in the warm season and a half in the cold season; in practice, they were collected each 80 samples per season (4 x 20 sample sets of incubation / number, corresponding to 80 samples / season).

Parameters analyzed were: Salmonella spp., Escherichia coli, Staphylococcus spp., Streptococcus spp. Using national working methods, standardized, effective and accredited RENAR. Detection and isolation of bacterial strains was made on liquid and solid culture media for pre-enrichment, enrichment, isolation and biochemical identification. They were used to confirm and galleries mini-API specific to each category of bacteria partly namely Salmonella spp. and Escherichia coli - ID Galleries 32 E; Staphylococcus spp - ID 32 STAPH galleries and Streptococcus spp - rapid ID 32 Strep.

From a total of 160 samples analyzed in summer, the two hatcheries, resulted in a 19.37% percentage of contaminated samples.

In winter I examined all 160 samples of same hatcheries and resulted in a rate of 1.25% of contaminated samples.

From analyzes revealed that embryonated eggs were contaminated with the same types of bacterial strains, which are Escherichia coli and Staphylococcus spp., incubation of both stations.

The highest incidence was Escherichia coli strain in a percentage of 13.75%; and Staphylococcus spp strains were found at a rate of 5.62%.

Key words: isolation, identification, bacterial strains embryonated eggs

INTRODUCTION

Embryonic mortality and survival rate of chicks post hatching depends to an extent significant degree of contamination of eggs for hatching and especially the pathogenicity strains contaminants, which is why we decided to establish the degree of contamination of eggs incubation [2].

Preventing disease in poultry requires a complex biosecurity, vaccination and hygiene especially at [1 and 2].

It can be said that there is a hierarchy of measures for prevention of diseases that could be: hygiene and decontamination, immunization, maintenance, operation and medication [3].

Most diseases are emerging from embryos due to vertical transmission, and in this way can transmit the following diseases: salmonellosis, respiratory mycoplasmosis, pseudomonoză avian colibacillosis, Newcastle disease, aspergillosis etc. [6].

Due to these diseases that can occur in farms of broiler chickens, we performed bacteriological analysis of embryonated eggs at the age of 18 days before hatching.

From this standpoint I tried to identify the degree of contamination of hatching eggs from two different seasons, summer and winter.

Samples were tested for bacteriological using standardized working methods and accredited RENAR.

Sampling was conducted in two hatcheries different units of growth of broiler chickens.

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MATERIAL AND METHOD

The study was conducted on a total of 320 samples of various incubation units every 80 samples / season. The period was divided into two seasons: one summer (June-August 2015) and one winter (December 2015-February 2016). The biological material consisted of chicken embryos aged 18 days.

Each season was split into 4 series of incubation; each series were harvested from embryonated eggs by 20 test under 18th day of incubation.

The biological material consisted of chicken embryos aged 18 days following that after hatching, day-old chicks populate the broiler farms. These samples come from its own hatchery each unit.

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

1. Step primary isolation - is to stimulate the growth of bacterial strains on selective agar medium and nutrient broth usual.

After sowing the two media I thermostat at 37°C for 24 hours.

2. Step of identifying - in the bird comprises the two areas from the previous step, the isolation medium selective, depending on the characteristics of the development of bacterial cultures.

3. Identifying and confirmatory biochemical tests miniAPI.

To isolate and identify the bacteria of the genus *Salmonella spp*., We have seeded the liquid medium - nutrient broth on selective isolation medium AgarXLD (xylose-lysine-deoxycholate agar). I thermostated at 37°C for 24 hours. I read plates after this period, we found no microbial growth and development of specific bacteria of the genus *Salmonella spp.*

To isolate and identify the bacteria of the genus *Escherichia coli*, we seeded environments usual broth and nutrient agar, special media diagnostic group *Enterobacteriaceaelor*, noting that the nutrient broth we noticed a haze intense ring surface and nutrient agar were developed variable-sized colonies (2-6 mm diameter), opaque, pigmented, type S. Cultures emits an odor of ammonia.

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

To confirm the biochemical characteristics of these bacteria are ferment lactose and glucose, lysine decarboxylase produce is indole positive, do not produce hydrogen sulphide and ammonium citrate used as the sole source of carbon. For confirmation as safe as I used miniAPI ID Galleries 32 E.

For isolation and identification of bacteria of the genus *Staphylococcus spp* I seeded the usual broth and nutrient agar media. On the nutrient broth, staphylococci grow profusely, with a uniform deposit turbidity easily homogenized, while the environment is clear.

Commonly, the annular film forming surface. Solid-surface of the medium on nutrient agar, staphylococci formed in 24 hours in aerobic conditions, colonies with a diameter of up to 3 mm, and within 4-5 days their size is 3 to 10 mm.

These features development of colonies have passed the special environment of isolation Baird-Parker RPF.

On this medium for the selective isolation they have been developed black-gray colonies surrounded by a halo opaque.

To confirm the biochemical galleries using mini API ID 32 Staph.

For the isolation and identification of bacteria of the genus *Streptococcus spp*., The nutrient broth was passed from the selective isolation Edward agar.

The appearance of colonies on this medium are small, fine gray metallic luster, but I found microbial growth.

RESULTS AND DISCUSSIONS

Under the legislation, analyzed parameters are: *Salmonella spp., Escherichia coli, Staphylococcus spp. and Streptococcus spp.*

After performing these analyzes, we found that both units were embryonated egg contamination with E. coli and *Staphylococcus* bacteria (fig. 1 and 2).

Fig. 1 Colonies typical *E. coli* XLD agar

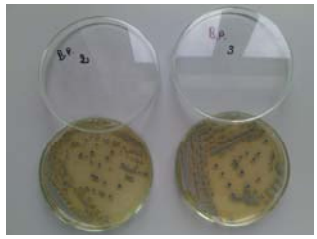

Fig. 2 Environment Baird Parker - typical colonies of *Staphylococcus spp*

Due to the development of isolation colonies typical environments, we conducted and galleries miniAPI (fig. 3, 4).

Biochemical characteristics of the bacteria of *E. coli* are they ferment lactose and glucose, producing lysine decarboxylase, is indole positive, does not produce hydrogen sulfide and ammonia does not use citrate as a sole carbon source (Figure 3).

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

Biochemical characteristics of the bacteria of the genus *Staphylococcus* are positive they are lactose, glucose, trehalose and mannitol; catalase activity presents obvious that enables differentiation of streptococci, who are catalase negative (Fig. 4).

Fig. 4 Identify Staphylococcus spp using API ID 32 galleries STAPH

Of the 320 samples of embryonated eggs, analyzed during the two seasons of the two hatcheries, we obtained the following results:

"UNIT A"

Of the 80 samples analyzed in summer we have a 85% were negative and 15% were positive, of which 12.5% positive E. coli and 2.5% positive *Staphyloccoc spp*. (table 1).

In the winter season from a total of 80 samples analyzed were all negative.

"UNIT B"

A percentage of 52.5% of the 40 samples analyzed in summer were negative, and 47.5% were positive, 30% positive samples in which *E. coli* and 17.5% positive samples in *Staphyloccoc spp*. (table 2).

In the winter season of the 40 samples analyzed, some 95% were negative, and 5% of samples were positive for *E. coli*.

Table 1 Results obtained in the embryonated egg at 18 days of incubation of the "A"

harvest time	Nr. samples analyzed	Nr. negative evidence				Nr. positive samples			
		Salmo-nella spp	E.coli	Staphy-lococcus spp	Strepto-coccus spp.	Salmo-nella spp	E. coli	Staphy-lococcus spp	Strepto-coccus spp.
summer 2015	80	80	70	78	80	-	10	2	-
winter 2015	80	80	80	80	80	-	-	-	-

Table 2 Results obtained in the embryonated egg at 18 days of incubation of the "B"

harvest time	Nr. samples analyzed	Nr. negative evidence				Nr. positive samples			
		Salmo-nella spp	*E.coli*	*Staphy-lococcus spp*	*Strepto-coccus spp.*	Salmo-nella spp	*E. coli*	*Staphy-lococcus spp*	*Strepto-coccus spp.*
summer 2015	80	80	68	73	80	-	12	7	-
winter 2015	80	80	78	80	80	-	2	-	-

CONCLUSIONS

From analyzes conducted we found that embryonated eggs were contaminated with the same bacterial strains, though samples come from two different hatcheries.

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

From the results also show that in summer, the degree of contamination of embryonated eggs was higher than in winter; one reason could be the difference in temperature between the two seasons, it is known that high temperatures favor the multiplication of microorganisms.

RECOMMENDATION

For the smooth running of the incubation process must be careful decontamination of incubators.

Hatching eggs must undergo a sanitation system to destroy microorganisms on their skin; handling hatching eggs made after disinfection of hands personnel, to prevent transmission of pathogens.

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