Cercetări Agronomice în Moldova
Vol. XLI , No. 2 (134) / 2008

RESEARCH ON INCUBATION BACTERIAL MICROFLORA

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Received January 11, 2008

ABSTRACT *– Investigations were conducted on samples of the pathological material, represented by 17-18 day embryonated eggs, unviable chickens that died in the first two days after hutching, and incubation wastes (eggshells). For germ isolation, inseminations were carried out on usual mediums (gelose and nutritive bullion) and on selective and enriching growth mediums (Chapmann, Drigalsky, Levin, Wilson-Blaire and Istrate-Meitert). The bacteriological exam allowed the isolation of a mixed flora, represented by Proteus vulgaris, Escherichia coli, Bacillus cereus, Staphylococcus aureus and Salmonella spp. In most cases, the germs were associated by two or three. Most of the strains were isolated from the strains of Escherichia coli - 82 (15.12%), Proteus vulgaris - 66 (12.36%), Staphylococcus aureus - 42 (7.93%), Bacillus cereus - 31 (5.72%) and Salmonella spp. - 5 (0.92%).*

Key words: bacterial flora, aerobe, incubation

REZUMAT - Cercetări privind flora bacteriană de incubație. *Cercetările s-au efectuat pe probe de material patologic, reprezentat de ouă cu embrioni de găină, în vârstă de 17-18 zile, pui neviabili, morți în primele două zile după ecloziune, şi deşeuri de incubație (cojile ouălor). Pentru izolarea germenilor, însămânțările s-au efectuat pe medii uzuale (geloză şi bulion nutritiv), dar şi pe medii selective şi de îmbogățire (Chapmann, Drigalsky, Levin, Wilson-Blaire, Istrate-Meitert). Examenul bacteriologic a permis izolarea unei flore mixte, condiționat patogene, reprezentată de Proteus vulgaris, Escherichia coli, Bacillus cereus, Staphylococcus aureus şi Salmonella spp. În majoritatea cazurilor, germenii erau asociați câte doi sau câte trei. Cele mai multe tulpini s-au izolat de tulpinile Escherichia coli - 82 (15,12%), Proteus vulgaris - 66 (12,36%), Staphylococcus aureus - 42 (7,93%), Bacillus cereus - 31 (5,72%) şi Salmonella spp. - 5 (0,92%).*

Cuvinte cheie: floră bacteriană, aerob, incubație

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INTRODUCTION

The death of embryos and of hen chickens at hutching is mainly caused by the incubation errors or the low egg quality. Other death factors are the infectious ones of endo- or exogenous nature, leading to a high increase in embryos and chickens mortality at incubation. Thus, Kostakev (1963) shows the case of septicaemia produced by *Streptococcus faecalis* and *Streptococcus faecium* in hen embryos and chickens, after hutching. He assumes that the intestine is the primary source of infection, with the consecutive germ dissemination in ovary and oviduct. Broad et al. (1965) described the egg infections with *Proteus vulgaris*, *Alcaligenes faecalis* and *Pseudomonas fluorescens*, while Iesus et al. (1965) isolated *Escherichia coli* from eggs and their shell and determined their serological type (Perianu, 2003). Because in some egg series, sampled from hens bred under intensive system, introduced in incubator, a high rate of embryo mortality was noticed, our purpose was to investigate the aerobe bacterial flora.

MATERIALS AND METHODS

Investigations were conducted on 542 samples of pathological material, represented by 272 eggs with 17-18 day dead hen embryos, 135 unviable chickens, which died in the first two days after hutching, and 131 incubation wastes (eggshells).

For isolating the aerobe bacterial flora, inseminations were done from bone, liver, heart, nutritive sac and from head and neck oedema, on usual mediums (gelose and nutritive bullion), and on selective and enriching growth mediums (Chapmann, Drigalsky, Levin, Wilson-Blaire, Istrate-Meitert) (Mânzat Moga, 2003).

The identification of isolated germs was based on the morphological, cultural and biochemical traits.

RESULTS AND DISCUSSION

From the investigated material, we have isolated a mixed bacterial flora, made of 82 (15.12%) strains of *Escherichia coli*, 66 (12.36%) strains of *Proteus vulgaris*, 42 (7.93 %) strains of *Staphylococcus aureus*, 31 (5.72%) strains of *Bacillus cereus* and 5 (0.92%) strains of *Salmonella spp.* (*Table 1*).

The obtained results showed that in the studied pathological material, the aerobe pathogenic bacterial flora was prevalent, which was represented by *Escherichia coli, Proteus vulgaris* and *Staphylococcus aureus, Bacillus cereus*, but also by potentially pathogenic germs of epidemiological importance, *Salmonella spp*. Because most of the embryos showed oedemas in the region of head and neck, lesions specific to the E avitaminosis or deficit metabolizations, we consider that isolations were done from a biological material lacking vitamins.

Table 1 - Results of the bacteriological exam

Studied material	Number of samples		*Proteus vulgaris*		*Escherichia coli*		*Staphylococcus aureus*		*Bacillus cereus*		*Salmonella spp.*	
	No	%	No	%	No	%	No	%	No	%	No	%
Embryonated eggs	272	52.03	28	10.29	31	11.38	18	6.61	15	5.55	3	1.10
Unviable chickens after hutching	135	24.92	21	15.55	22	16.21	15	11.11	5	3.70	0	0.00
Eggshells (wastes)	131	23.05	17	12.98	29	22.14	9	6.10	11	8.39	2	1.52
Total	542	100	66	12.36	82	15.12	31	5.90	31	5.72	5	0.92

The germs from *Proteus* genus could not be isolated from the first inseminations at pure state, being associated to *Escherichia coli* and *Bacillus cereus.* Compared to the biochemical tests (production of indol, H_2S and fermentation of maltose, mannitol, lyxosazone, inositol and sucrose), a single species behaved as belonging to the *Proteus vulgaris* species, the other strains being unframed in the Kauffman draft, from the biochemical viewpoint.

Because we have isolated from some embryos an associated flora, made of *Escherichia coli* and *Proteus vulgaris*, or *Bacillus cereus*, it was possible that the association of two or three pathogenic conditioned germs on vitamin lacking organisms, should result in increasing the infesting germ capacity and therefore, in their high multiplying in embryos (Bergdoll, 1998). An important role in transmitting the pathogen traits was due to some toxic components elaborated by germs from *Proteus* genus, which would favour the potentially infesting qualities of *Bacillus cereus* and *Escherichia coli*.

The obtained results have shown, besides the epidemiologic interest, a social importance of public health, because they signalled the frequent isolation of *Bacillus cereus* and *Proteus vulgaris* from eggs, germs known as toxi-infectious factors of food in humans (Bârzoi et al., 1999).

We could not establish the origin of the infection in embryos or eggs, which seemed to be exogenous, because the attempts of isolating the above-mentioned species from the ovary or oviduct of adult hens remained without any results.

At the necropsy of the dead embryos in the second period of embryogenesis (after 17 days) and of the unviable ones (1-2 days after hatching), we noticed lesions of omphalitis, congestions, serous or serous-fibrinous exudates on the

toracal serous leaves, blood effusion in heart and liver and urate accumulation in cloaca. In transovarian and organoleptic infested embryos, the calf was yellow-brownish coloured, of caseous or aqueous aspect. The vitellin sac was congestioned and oedema like.

CONCLUSIONS

The bacteriological exam of the 542 samples of pathological material, represented by dead hen embryos, unviable chickens and incubation wastes (eggshells), led to the isolation of a mixed aerobe bacterial flora made of *Proteus vulgaris, Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Salmonella spp.*

In most of cases, the germs were associated by two or three. The most frequently found association was *Bacillus cereus* + *Proteus*, *Escherichia coli* + *Proteus vulgaris* şi *Escherichia coli* + *Proteus vulgaris* + *Bacillus cereus*.

The greatest number of strains was isolated from strains of *Escherichia coli* - 82 (15.12%), followed by *Proteus vulgaris* - 66 (12.36%), *Staphylococcus aureus* - 42 (7.93%), *Bacillus cereus* - 31 (5.72%) and *Salmonella spp.* - 5 (0.92%).

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