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FACULTY OF VETERINARY MEDICINE
DOMAIN VETERINARY MEDICINE
SPECIALITY INFECTIONS DISEASE

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DOCTORAL THESIS

Scientific leader

Prof. Univ. Dr. Tudor Perianu,

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RESEARCHES REGARDING THE INFECTIOUS PATHOLOGICAL OF INCUBATION

These for obtaing scintific title
„Doctor in veterinary medicine"

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Summary

The eggs and the fowl meat are food with numerous qualities that makes that in the future their production to rise constantly, fact that is results also from the data published in the specialized international magazines, of high reputation. Thus, their dietetically qualities make them useful in various diseases and in the convalescence period, and their high biological-trophical level, as well as the acceptance of their usage by all the people, no matter the religion, highly recommends them for being consumed without any restriction. That's why in the following years, the egg and poultry breeding production will be extremely spectacular, in some of the areas. According to the predictions in the "Poultry international", in the year 2010, in the USA, there will be consumed 500 eggs by inhabitant, and in 2040, 85 kg of poultry, out of which 27 kg turkey meat.

The rent ability in the poultry breeding area depends on the achieving possibilities of a high meat and egg production with a minimum of expenses. In the breeding and raising the productive poultry, the science and the poultry breeding practice have appealed to semi industrial first, and then industrial methods.

By incubation we mean he development process of the embryo with latency life within the fertilized egg till the obtaining/ acquiring/getting a one day old chicken or a duckling, with normal viability, under the influence of some physical factors, in a certain period of time, according to each species or even poultry breed.

The eggs' incubation can be natural, when the necessary physical factors for the embryonic development are ensured by the clucking/brood hen and by the artificial one, when these factors are ensured by the incubators.

The fresh eggs are very resistant to the microbial contamination, under the condition that come from healthy poultry, fed reasonably, sheltered in hygienically conditions and who benefit from an appropriate microclimate.

A higher danger risk level for the embryo's life is represented by the microorganisms responsible for the starting of an infectious disease and which are transmitted directly from the ill poultry or formerly ill to the life core from the in the germinative disc of the eggs.

The special resistance to the microbial contamination of the fresh eggs is given, mainly by the membranes' presence which protects the yolk of the egg - the most vulnerable structural component of the eggs to the microbial attack. In addition, the lisozime contained within the content of the white of the egg, exerts a strong bactericide action. Unlike the fresh eggs, the old

ones kept inappropriately are improper t only for the incubation, but also for the large consuming, being easily contaminated by the microorganisms in the environment medium.

A higher risk level of danger for the embryo's life is represented by the microorganisms responsible for the starting of an infectious disease and that are transmitted directly from the ill poultry or been ill from the in the germinative disc of the eggs.

The main infections sources and implicitly of the embryos that grow within these eggs are ill birds and those been ill, that eliminate for a longtime the germs through excrement, thus determining the contamination of the shelters, holes, water and the nourishment.

In the work paper it has been followed the research of the aerobe bacterial flora that can influence the eclosion percentage, in natural conditions as well as artificial. But also the pathogenesis of the isolated germs and identified.

The paper includes 167 pages, being typed in IX chapters and is structured in 2 parts. The first part (Chap.,I, II and III) is synthesizing the main bibliographical data in the specialized literature regarding the incubation aerobe bacteria infections and constitutes the "Knowledge stadium" and the second part (cap.IV, V, VI, VII and VIII) are referring to personal research. Each chapter in the second part of the thesis has in the content material and working methods, the obtained results with their discussion and the partial conclusions. In chapter IX there are synthesized in the 29 final conclusions, the main aspects drawn from the made research.

In the work there are exhibited 21 tables, 95 figure and the bibliography is including 130 titles.

The first part of the paper, is representing a synthesis of the literature regarding the etiology of the incubation infectious pathology, with accent on the morphology the incubated egg, of the factors who are influencing the egg quality, as well as the actual stadium on the international level, of the research made for the scientific research of the action of the pathogens agents with bacterial nature, on the egg and on embryo.

In chapter one, there are presented the available data related to the morphology of the incubation egg.

Chapter II is treating the factors that influence the quality the incubated egg before deposition, after it has been deposited, as well as the factors that influence the incubation process. There have been reviewed the mating methods and the sexes report.

Chapter II, presents the etiology of the incubation infections. There are presented the infections produced by the microorganisms pathogenically conditioned (Proteus spp., Staphylococcus aureus, Escherichia coli), as well as the infections determined by pathogens microorganisms, Salmonella spp. (tifo- pulorozis şi paratiphosis aviare).

The premisses from which they started performing their own researched were those to emphasize some particularities of the bacteria infections in conditions of artificial incubation, as well as some aspects of the incubation process in natural conditions on different poultry breeds (hens or ducks).

In pat 2 “Own researches”, constructed in V chapters, the investigations followed the aerobian bacterial flora of hen and duck eggs before being incubated (cap. IV) “the aerobian bacterial flora of the incubated eggs gathered from poultry raised in intensive system (cap. V) , “aerobian bacterial flora of the incubated eggs (the clucking hen), gathered from fowls raised in an extensive system , householded” (cap.VI) , “the antibacterial activity of some chemico-therapeutics over the isolated aerobian bacteria stems” (cap.VII) and the “ surveilling and preventing strategy of the incubator aerobian bacterian infections” (cap.VIII).

In chapter IX, there are synthesized: “the final conclusions “resulted after the bacteria researches, regarding the incubating aerobe bacteria infections.

The researches regarding the bacterial flora of the egg on a number of 547 egg out of which 290 hen and 257 duck, 24 hours old (125, 165 respectively) and 20 (170, 87 respectively) days old from the laying, n incubated (rmal), led to the isolation of 67 aerobe stems, that means 12,24%.

The isolated aerobe bacteria flora was identified as belonging to the species: Bacillus cereus 20 (3,66%) stems, Staphylococcus aureus 14 (2,38%) stems, Escherichia coli 13 (2,35 %) stems, Proteus vulgaris 12 (2,17 %) stems and Salmonella spp. 8 (1,46 %) stems. We can observe that, although the egg benefits of means of natural protection (shell, shell membranes, the cuticle, as well as the richness of the white of the egg in the lizozime), still exposed to the invasion by different microorganisms. The aerobe bacteria proportion present in the structural components (the yolk of the egg, the white of the egg, with 24 hours old from laying and 14,40-25,08% for those 20 days old, kept in ordinary temperature and humidity conditions.

The isolated aerobe bacteria flora is different according to the structural component, to the length of days and the eggs’ origin (hen or duck). Thus, as far as the structural component is concerned, we can observe that the white of the egg of 547 hen and duck eggs , bacterial stem has been isolated , while from the yolk of the egg it has been isolated 1 stem of Salmonella spp. (0,58%) from the duck eggs.

From the fresh hen eggs it have been isolated 7 (11,20%) stems, and over the shell of those 20 days old, a number of 16 (9,60 stems). In exchange, over the shell of the fresh duck eggs there have been isolated 13(7,54) stems of aerobe bacteria and 16 (18,24%) over the shell of the old duck eggs.

The presence of different percentage of isolated germs from the shell, explains the excrement soiling degree of the egg, as a result of the different laying way, different from the hen, of the humidity degree of the surface and the depositing and keeping conditions.

The results obtained, present, besides the epidemiological interest and a social importance, of public health, as it signals/draws attention the frequent isolation of Bacillus cereus şi Proteus vulgaris in the eggs, germs kwn as potentially toxi-infectious factors of alimentary nature to the humans.

The researches regarding the incidence of the aerobe bacterial infections of the eggs introduces into incubation, gathered from poultry raised in intensive system (chapter 5) have been made during 2005-2007, on 281 samples of pathological material, represented by 132 hen eggs with dead embryos 17-18 days old, 76 n-viable poultry dead after the eclosion/being laid, 1-2 days old and 73 incubation wastes (eggs' shells).

The bacteriological exam has led to the isolation and identification of 178 aerobe bacteria stems, that is 63,10 %.

The isolated aerobe bacteria flora is represented by the bacteria species: Escherichia coli 52 (18,81%) stems, Proteus vulgaris 46 (16,37%), stems, Staphylococcus aureus 27 (9,60%) stems, Bacillus cereus 27 (9,60%) stems, Streptococcus spp. 13 (4,66%) stems şi Salmonella spp. 3 (1,06%) stems.

From the obtained results we can observe that in the pathological material examined, prevails an aerobe bacteria flora pathogenically conditioned, represented by: Escherichia coli, Proteus vulgaris, Bacillus cereus, Staphylococcus aureus and Streptococcus spp., but also by germs potentially pathogens of epidemiological importance, Salmonella spp. Thus, the 3 stems of Salmonella 2 (66,66%) of them were isolated izolat (66,66%) stems on the egg shells after the laying (wastes) and 1 (33,34%) of them from the embryos. The isolated stems biochemically and serologically, have been identified as belonging to the species: Salmonella djugu 2 (66,66%) stems şi Salmonella typhimurium 1 (33,34 %) stem.

To be mentioned that Salmonella typhimurium, generally considered, as a result of its great adaptation capacity, the most spread and as a predominant serotype, only one stem gas been isolated, that is 33,34 %.

The bacteria researches regarding the aerobe bacteria infections of the hen and duck embryos, resulted form the incubated eggs in extensively- household system (to the clucking/brood hen) (cap.VI), gave led to the isolation of 108 (42,67% of aerobe bacteria stems, most of them pathogenically conditioned, represented by the genres: Bacillus, Proteus, Escherichia, Staphylococcus, Streptococcus şi Salmonella.

The isolated aerobe bacteria stems differ according to the origin of the researched material. Hence, out of 127 pathologically material gathered from the embryo hen eggs 42 of them have been isolated and identified (33,14%), and of 126 samples of pathological material originated from the duck embryo eggs, 67 stems have been isolated, representing 53,17%.

The isolated bacteria species differ according to the origin of the pathological material. This way, the samples that came from the hen embryo eggs Escherichia coli, Proteus vulgaris şi Stahpylococcus aureus, have been isolated and of those gathered from the duck eggs Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Streptococcus spp. and Salmonella spp.

The isolated germs, in most of the cases were associated by two or by three, more frequently finding the association Bacillus cereus – Proteus vulgaris or Escherichia coli – Proteus vulgaris – Bacillus cereus.

In chapter VII, named "researches regarding the antibacterial activity of some chemo-therapeutics to some of the aerobe bacteria stems", it has been investigated "in vitro" the activity of 10 substances antibacterial, as opposed to 226 stems isolated between 2002-1007 from different pathological material (embryo hen eggs, n viable hen chicken , incubation wastes).

The obtained results have pointed out that the antibacterial activity towards the aerobe bacteria flora differs according to the used substances. In this way, the most active have proved to be gentamicine (89,78%) , followed in countdown by order , amoxiciline (87,61%), kanamicine şi furazolidone with (87,17%), ampiciline (79,38%), cloramfenicol (78,31%), eritromicine (34,51%), tetracicline 28,87%, colistin (28,87%) şi streptomicine (8,84%). The streptomicine, colistinul , tetracicline and eritromicine proved themselves less active, most of the isolated stems (42,47 – 77,45%) being resistant. Also, the bacteria activity of the researched substances differs also according to the examined bacteria species. This way, the Proteus vulgaris proved very sensitive to the kanamicine action (78,78%), gentamicine (72,28%), furazolidon (56,07%) and ampiciline (68,78%), sensitive to streptomicine (39,39 %) and eritromicine (37,39%) and resistant to the tetracycline activity (53,02%) and cloramfenicole (48,50%).

Escherichia coli proved very sensitive to the gentamicine's action (71,96 %), kanamicine (66,10 %), colistin (63,67 %) and furazolidon (52,43 %); sensitive to the activity of cloramfenicol (37,81 %) şi amoxiciline (29,39 %); resistant to the activity of tetracicline (63,67 %), eritromicine (62,21 %), streptomicine (57,33 %) and ampiciline (52,43 %).

Staphylococcus aureus very sensitive to gentamicine (97,62 %), amoxicinline (95,24 %), ampiciline (83,34 %) and kanamicine (83,24 %); sensitive to furazolidon (53,62 %) şi colistin (30,94 %) ; resistant to streptomicine (64,48 %) and cloramfenicol (57,16 %).

Bacillus cereus proved very sensitive on amoxiciline (93,56%), kanamicine (87,12 %), gentamicine (83,90%) and ampiciline 54,00%); sensitive to the action of the tetracicline action (58,14 %) and colistin (41,86%) resistant 100% to the action of eritromicine (41,86%) and cloramfenicol (38,64 %), and Salmonella spp. Being very sensitive to the activity of amoxiciline 4(80,00%), gentamicine (80,00%), ampiciline (60,00%), kanamicine (60,00%) and furazolidon (60,00%), sensitive to tetracicline (80,00%) and cloramfenicol (60,00%), resistant to the activity of amoxiciline, tetracicline and eritromicine 100%.

The sensitivity of the isolated aerobe bacteria flora is obvious on the chemico-therapeutics that were used on a small scale or at all in profilactico-curative purposes or as bio stimulator in feeding the pultry.

Through strictly applying and respecting the strategically surveillance and prevention measures of the incubating bacteria infections (Chapter VIII), there has been obtained the percentage diminishing of the n-fecundated eggs (1,8% 0,9%), as well as of the dead embryos from 8,41% to 7,8%. The percentage of viable chicken has risen from 63,11% to 77,90 %.