ETHIOLOGICAL STRUCTURE AND EPIDEMIOLOGICAL MONITORING OF BACTERIAL INFECTIONS IN INDUSTRIALLY REARED BIRDS IN BULGARIA*

ETIOLOŠKA SRUKTURA I EPIDEMIOLIŠKI NADZOR BAKTERIJSKIH INFEKCIJA KOD INDUSTRIJSKI GAJENE ŽIVINE U BUGARSKOJ

Valentina Urumova, M. Lyutskanov

The study was performed on the 9 largest poultry farms and hatcheries in Bulgaria during the period from 2001 to 2006.

Eight hundred thirty three samples from birds have been investigated – seven hundred ten from gallinaceous and one hundred twenty three from waterfowl. The samples have been taken from corpse material (liver, spleen, joint, air sack, small intestine) as well from dead embryo, cloacal and nasal swabs. They were analyzed using routine laboratory or the semi-automatic system of identification CRYSTAL for enterobacteriae and staphylococci, as well as using the API-20 NE system for nonfermentative bacteria.

A positive microbial finding was current in 67.8% of samples from dead chick embryos. The isolates belong to 13 different microbial species. A total of 14 species were isolated from the corpse material, nasal and cloacal swabs. Their specific presence is age dependent. Similar results have been found for waterfowl.

Key words: ethiology, bacterial diseases, reared birds, routine methods, diagnostic

Introduction / Uvod

Bacterial diseases account for a considerable share of infectious pathology in intensively reared birds. Their successful control requires a profound knowledge of their etiological structure, especially the dynamics, as well as their

* Rad primijen za štampu 12. 7. 2007. godine
** Valentina Urumova, Mihni Lyutskanov, Department of Veterinary Microbiology, Infectious and Parasitic Dyseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria
epidemiological features. For some microbial species there is a real problem of establishing routine methods of diagnostics.

The aim of the present study was to elucidate the etiological structure and the dynamics of manifestation of different bacterial infections in industrially reared birds – poultry and waterfowl, in some of the big farms in Bulgaria with regard to the optimization of prevention and control procedures.

Materials and methods / Materijal i metode ispitivanja

The epidemiological studies were performed on 9 farms – 6 poultry farms and 3 waterfowl farms using various rearing technologies.

A total of 833 samples were analyzed, including 710 from gallinaceous birds and 123 from waterfowl. The samples were obtained from dead embryos, from newly hatched chickens and ducklings, as well as from growing birds at a different age in order to monitor the dynamics of the etiological structure.

In order to determine the etiological factor, bacteriological investigations were carried out in samples as follows:

– Dead embryo samples – every sample covered 12 embryos; lung tissues and air sack.

– Corpse material samples – every sample covered 6 corpses.

From each corpse ventricle blood, liver, spleen, marrow, and in case of arthritis a joint sample were investigated. Lung tissue samples and air sack samples were taken in case of indication of mycoplasma. Lung tissue samples were taken in case of indication of ornitobacteria. Small intestine samples were investigated in case of indication of clostridia.

Bacteriological investigations also included:

– Cloacal samples received from chickens and fowls originating from the same farms.

The samples were bacteriologically analyzed using routine laboratory techniques for isolation, identification and typization. A part of the microbial isolates were identified by the semi-automatic system of identification CRYSTAL for enterobacteriae and staphylococci, as well as via using API-20 NE system.

Results / Rezultati

The bacteriological analysis of 355 samples from dead chick embryos revealed a positive microbial finding in 241 (67.8%) samples whereas no bacteria were present in 32.3 % of samples.

The obtained isolates included representatives of 13 microbial species (Fig. 1). Most commonly, representatives of the Enterobacteiraeae family, headed by *Escherichia coli* (23.2%) were isolated from dead chick embryos. This
was valid for all surveyed flocks. The second prevalence was that of the *Proteus* genus (16.1%). Members of *Enterobacter* spp. were found in 5.7% of samples. The other enterobacteriae included *Klebsiella* spp. and *Citrobacter* spp.

Gram positive cocci (staphylococci, streptococci and enterococci) were recovered relatively rarely – only in single samples. *Ps. aeruginosa* isolates were detected considerably more frequently – this organism was evidenced in 4.5% of samples.

**Bacteriological findings in newly hatched broiler chickens (0-10 days of age) / Bakteriološki nalaz kod tek izleglih brojler pilića (starosti 0-10 dana)**

The bacteriological investigations of 171 carcasses of broilers chickens at the age of 1 - 10 days, originating from 5 different farms, yielded findings including members of 9 various microbial species, shown on the figure 2.

Again, in newly hatched chickens, *Escherichia coli* was detected in 36.8% of studied samples. Similarly to the picture with chick embryos, the second place was held by representatives of the *Proteus* genus (16.8%).

The third prevalence was that of *Salmonella* spp. representatives, observed in 7.0% of samples. As the cause of death in newly hatched broiler chickens, members of *Enterobacter* spp. and *Ps. aeruginosa*, were next detected in 4.7% and 4.1% respectively if studied carcasses, but not in all studied batches.
With the increase of age, the relative proportion of samples with a positive bacterial finding decreased. Sterile samples accounted for 72.9%. The microbial isolates in the other 27.1% consisted of representatives of 6 species. The most commonly isolated species in this age group was again *E. coli*, present in 20.6% of studied samples.

Figure 2. / Slika 2.

**Bacteriological findings in broiler chickens at the age of 10 - 30 days / Bakteriološki nalaz kod brojler pilića starih 10-30 dana**

With the increase of age, the relative proportion of samples with a positive bacterial finding decreased. Sterile samples accounted for 72.9%. The microbial isolates in the other 27.1% consisted of representatives of 6 species. The most commonly isolated species in this age group was again *E. coli*, present in 20.6% of studied samples.

Figure 3. Percentages of bacterial isolates from dead broiler chickens at the age of 10-30 days / Slika 3. Procenat bakterijskih izolata kod uginulih brojler pilića starih 10-30 dana
An interesting finding in this age group was the detection of anaerobic bacteria from the *Clostridium genus* (4.7%). In this case, there was an outbreak of necrotic enteritis in 3 different batches in one farm and in a single batch from an other farm. The other microbial species, including the salmonellae observed in 3 carcasses from one farm, could be considered as accidental.

**Bacteriological findings in broiler chickens older than 30 days / Bakteriološki nalaz kod brojler pilića starijih od 30 dana**

In broiler chickens older than 30 days, the species diversity of bacteria continued to decrease. Four microbial species were evidenced, among them *Mycoplasma spp.* This was the typical age for infection of birds with mycoplasmae and as expected, they were associated with colibacteria.

![Figure 4. Percentages of bacterial isolates from dead broiler chickens older than 30 days / Slika 4. Procenat bakterijskih izolata kod uginulih brojler pilića starijih od 30 dana](image)

**Bacteriological findings in newly hatched ducklings (0-7 days of age) / Bakteriološki nalaz kod tek izleglih pačića (starosti 0-7 dana)**

The bacteriological analysis of samples obtained from dead ducklings during the first 7 post hatching days showed isolates belonging to 7 microbial species (Fig. 5).

It could be seen that 55.6% of carcasses contained bacteria that could be determined as etiological agents.
Similarly to broiler chickens, *Escherichia coli* was the most prevalent species in newly hatched ducklings – in 21.6% of samples. Then followed species from the *Proteus-Providentia-Morganella* group. The greatest part of isolates were from carcasses of just hatched ducklings and birds up to the age of 3 days, suggesting their vertical transmission.

The more frequent detection of *Ps. aeruginosa* (7.5%) should be emphasized – a twofold higher percentage compared to the findings in newly hatched broiler chickens at the same age. Furthermore, the data were almost comparable for all farms surveyed.

**Bacteriological findings in growing ducklings (7-21 days of age)** / **Bakteriološki nalaz kod pačića u rastu (starosti 7-21 dana)**

For this age group, the microbial findings included members of 5 microbial species, presented in Fig. 6.

In growing ducklings, the species diversity of bacterial etiological agents decreased compared to that in newly hatched ones. Again, *Escherichia coli* (18.8%) was dominant, although compared to the first age group, its relative proportion was reduced. The share of salmonella-induced infections, although not high (12.6%), deserved attention because of the high lethality and the risk of a permanent carriership. Special attention should be paid to the isolation of 4 samples of *Riemerella anatipestifer*, the agent of the bacterial polyserositis. The infection was found in 5 batches of different origin in 3 different farms.
The analyses proved the existence of 3 microbial species: *Pasteurella multocida* (18.2%), *Salmonella Typhimurium* (9.1%) and *Riemerella anatipestifer* (9.1%).

The detection of *Pasteurella multocida* causing avian cholera was not unusual in waterfowl rearing whereas the presence of *Riemerella anatipestifer* confirmed the increasing importance of this type of infection. This was also accentuated by the fact that the agent was isolated also from 4 rats from the affected farm. Up to now, similar data were not available in our country.

**Reviewing and conclusions**

1. Bacterial infections occupy an important place in the pathology of industrially reared birds – both chicken and waterfowl species. A significant species diversity of etiological agents, most obvious in dead chick embryos and newly hatched chickens and ducklings, was established.

2. With the advancing of age, the number of encountered microbial species in chickens and waterfowl decreased and this was valid for all studied farms and flocks.

3. In chicken fowl at all ages, the *Escherichia coli* sp., causing embryonal death and severe septicaemiae in newly hatched and growing chickens and local infections in older birds, was absolutely dominant.

4. The frequent isolation of *Proteus-Providentia-Morganella* (PPM), whose role in chick embryo pathology is known, implied a revision of the ap-
proach to them throughout their isolation from newly hatched and growing chicks. This was generally applicable also for *Ps. aeruginosa* related infections.

5. In growing chickens, clostridial and mycoplasmatic infections were recognized as problematical.

6. The investigations did not confirm the anticipated more frequent isolation of microbial species as *O. rhinotracheale*, *Bordetella avium*, *Kingella kingii*, *Pasteurella gallinarum*, but this could be due to the relatively low number of tested farms and the rather short period of the survey.

7. In ducks, the repeated detection of pasteurellae as well as of the *Riemerella anatipestifer* species deserved special attention. The isolation of the latter from rats was also an important epidemiological finding with regard to the possibility for the formation of carriership in rodents, combined with accidental or even permanent shedding.

References / Literatura

Ispitano je ukupno 833 uzoraka živine – 710 uzoraka živine i 123 uzoraka vodenih ptica. Uzorci su uzeti sa leševa (jetra, slezina, zglob, vazdušne kese, tankog creva), kao i sa uginulih embriona, brisevi sa kloake i iz nosa. Analize su vršene koristeći rutinske laboratorijske metode ili polu-automatski sistem identifikacije CRYSTAL za enterobakterije i stafilokoke, kao i API-20 NE sistem za nefermentne bakterije.


Ključne reči: etiologija, bakterijske bolesti, gajene ptice, rutinske metode, dijagnostika