Cultural, biochemical, and pathogenic properties of Escherichia coli isolated from birds

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Abstract. The article presents data on the study of cultural-biochemical, tinctorial, biological, and antigenic properties of pathogenic Escherichia coli, isolated from the pathological material of chickens and dead from Escherichiosis, by conducting bacteriological and serological studies in some poultry farms in the Republic of Uzbekistan.

1 Introduction

Escherichiosis (colibacillosis) occupies one of the leading positions in the infectious pathology of animals and birds; it accounts for about 50–60% of the mortality of poultry [2]. The economic damage caused by the disease is very significant and consists of a decrease in egg production and weight gain, the deaths of embryos, chickens, and adult birds, and the cost of measures to improve the economy [3].

Eshirichiosis is a systemic infection caused by enteropathogenic strains of Escherichia coli, the main source of which is a sick and recovered bird. Transmission of the pathogen by wild birds and rodents is also possible. The most susceptible to infection are chickens aged 1–10 days, in which escherichiosis occurs in the form of acute sepsis. In chicks over the age of 10 days, the infection is characterized by pathoanatomical signs of serofibrinous or fibrinous pericarditis, perihepatitis, hemorrhagic duodenitis, and aerosacculitis. In birds that have had escherichiosis, escherichia coli can be localized on the intestinal mucosa, larynx, nasal cavity, and trachea [5]. Escherichiosis can occur as a secondary infection that occurs when the bird is immunosuppressed and also complicates the course of viral and bacterial diseases [4].

Escherichia coli is a permanent inhabitant of the intestinal tract of most warm-blooded animals and humans and is also often found in the environment. Currently, more than 150 different serovariants of Escherichia coli are known.
2 Relevance of the topic

In recent years, Uzbekistan has seen a stable development of the poultry industry and an increase in the number of poultry. At the same time, the concentration of a significant number of birds in limited areas is fraught with a serious risk of spreading infections in farms, such as escherichiosis. In Uzbekistan, avian escherichiosis is widespread on poultry farms and causes great damage to the poultry industry.

3 Purpose of research

To find out the cultural-biochemical, tinctorial, biological, and antigenic properties of pathogenic Escherichia coli, isolated from the pathological material of patients and those who died from Escherichiosis, by carrying out bacteriological, serological, and pathoanatomical studies.

4 Methods, material, methods and scope of research

To establish epizootic serotypes in laboratory and production conditions, 1815 blood samples of birds belonging to poultry farms in three regions of the republic were examined. To conduct epizootological monitoring of avian escherichiosis in poultry farms, the methods of clinical observation, pathoanatomical autopsy, epizootological analysis, and mandatory laboratory studies were used.

To grow cultures of Escherichia coli, MPB and MPA were used, and for the purpose of differentiation, inoculations were made on Endo or Levin medium, kept at 37 °C for 24 hours. Serum produced by the Armavir biofactory of the Russian Federation was used to set up the agglutination reaction (RA). Pathological material was seeded on BCH, MPA, and a Petri dish with Endo's or Levin's different medium. Sowing in a Petri dish was carried out from the spleen, liver, gallbladder, and bone marrow of birds. Two typical S-shaped colonies for Escherichia coli were sifted from Endo or Levin agar, and two tubes of MPA were used to prepare smears of inoculation on a differential diagnostic medium. The second tube was used to prepare an autoclaved antigen if the boiled one would not agglutinate with polyvalent coli sera. Morphological, tinctorial, cultural-biochemical, and pathogenic properties were studied in agar cultures.

To study the morphological properties, smears were stained according to Gram, and mobility was determined by the nature of growth in semi-liquid MPA. Cultural and biochemical properties were studied on a set of media, which included media with carbohydrates and Andrade indicators (lactose, glucose, sucrose, mannitol, dulcite, adonite, and inositol).

In studies conducted on various internal organs, especially the bone marrow of bird corpses, compound feed, feed additives (meat-bone and vitamin-grass meal and silkworm pupae), water, droppings, and suffocating embryos, we isolated Escherichia coli strains of various serotypes, among which were a significant number of pathogens for 3-week-old chickens and white mice. The total was allocated. 28 strains, of which 24 serogroups turned out to be pathogenic.

Escherichia coli are polymorphic, straight or slightly curved movable rods with rounded ends of medium size (length 2–6 microns and width 0.4–0.6 microns). Sticks are located singly or, less often, in pairs. A dispute does not form.

Escherichia coli are aerobes or facultative anaerobes. The optimum growth temperature is 35–37 °C. They grow well on simple nutrient media. On MPA, Escherichia coli form colonies of medium size, gray-white, smooth, moist, shiny, and with smooth edges (S-form).
In liquid media, they grow diffusely, cause uniform turbidity in the medium, and sometimes form an insignificant precipitate (less often, they form a surface film or a wall ring).

On Levin's medium, lactose-positive Escherichia coli strains formed dark purple colonies with a greenish tint.

Lactose-positive strains of Escherichia coli on Endo's medium form dark red colonies with a metallic sheen. Lactose-negative Escherichia coli, which do not ferment lactose, formed colorless or pale pink colonies on Endo's medium.

On the Endo medium, the growth of Escherichia coli was characterized by the formation of large columns with smooth edges, having a red or dark crimson color, often with a metallic sheen. Obtaining a pure culture was recognized by us after a three-fold transfer to MPA.

Escherichia coli bacteria stained negatively according to Gram, and most were motile. All studied strains of Escherichia coli coagulated milk, did not liquefy gelatin, gave a negative reaction with Voges-Proskauer, and the test with methylrot was positive. Escherichia were sticks with rounded ends. On the BCH, a uniform turbidity was observed, and sometimes a film was formed.

Escherichia coli had a high biochemical activity; they fermented with the formation of acid and gas, glucose, lactose, maltose, arabinose, galactose, and mannitol. Dulcite and sucrose were not fermented by most Escherichia coli strains.

All strains did not grow on Simmons medium. Some strains decomposed urea; all utilized sodium malonate; most fermented rhamnose, sucrose, mannitol, sorbitol, trehalose, dulcitol, and glucose; most strains formed indole and emitted hydrogen sulfide. (Table 1)

From the data in Table 1, it can be seen that when studying the biochemical properties of Escherichia coli strains, we found that the bacteria have high enzymatic activity. Carbohydrates: glucose, lactose, maltose, mannitol, rhamnose decomposes to the formation of acid and gas, i.e., these strains are typical of the genus Escherichia.

In an experiment conducted in the Samarkand region, pathological material from 20 45-day-old chickens was seeded on MPA, MPB, and Petri dishes with Endo and Levin differential medium (agar with eosin and methylene blue). Inoculations for MPA and MPB were performed with a Pasteur pipette. After 18–24 hours of incubation in a thermostat, the crops were examined and, in the absence of growth, were kept for another day. In those cases where there was no growth on the Endo medium and turbidity of the medium was noted in the BCH, microscopy was performed and the presence of growth was checked for.

Cultures obtained from at least two organs, including bone marrow, were subjected to the study. Typical colonies were round, smooth, with a slightly raised surface in the center; smooth edges of pink, red, or raspberry color with and without a metallic sheen on Endo's medium; and purple or black on Levin's medium.

Of the 93 cultures of Escherichia coli studied, lactose was degraded to acid and gas 81.9%, glucose was fermented 93%, sucrose was fermented 90%, and mannitol was decomposed 87.9%. Oxidized arabinose, maltose, galactose (100%), dulcitol and sorbitol (90%), and raffinose (30%) It was found that out of 93 Escherichia coli strains after 24-hour incubation at a temperature of 370 °C, 27 strains formed indole and 40 strains did not form indole or hydrogen sulfide. After 72 hours of incubation, an additional 21 strains produced indole, and five strains produced hydrogen sulfide.

The studied strains of Escherichia coli gave a positive Voges-Proskauer test, i.e., they did not form acetylarchbinol. Didn't change Simmons' medium and didn't grow on Coser's medium. From Escherichia coli strains, positive RA on glass was given with the following sera: O33-27 strains, O75-27, O128-22, O25-21, O119-20, O114-18, O18-17, and O26-16 strains.

Thus, the 93 studied Escherichia coli strains were biochemically active. Strains of Escherichia coli with typical biochemical properties were typical for their group.
As a result of serological studies, it was found that the studied strains of Escherichia coli had a complex antigenic structure, which was confirmed by positive RA with 3, 6, 8, or more sera. In poultry farms in three regions, pathoanatomical changes were studied in dead birds from escherichiosis of different ages. As a rule, many noted exhaustion. During the autopsy of the corpses of chickens aged from several hours to 7–10 days, changes characteristic of septic diseases were established. At the autopsy of dead birds aged 11–99 days, fibrinous overlays were noted on the pericardium, epicardium, liver capsule, and in the air sacs. In adults, yolk peritonitis, catarrhal-fibrinous salpingitis, and atrophy of the ovary and oviduct were noted. During bacteriological studies of pathological material, 27 cultures of Escherichia coli were isolated, including 7 cultures from chickens, 10 chickens, and 10 chicken embryos. The results of serotyping Escherichia coli strains in RA on glass are presented in Table 1.

As can be seen from the data in Table 1, some strains gave positive reactions to several sera. After preliminary typing of Escherichia strains with type-specific sera in RA on glass, test-tube RAs were placed, according to the results of which the first strain, which gave positive reactions with 17 sera, was assigned to the O26 serotype (three crosses in a dilution of 1:6400). the second strain, which gave positive reactions with 10 sera, to the O55 serotype (three crosses in a dilution of 1:3200). The third strain, which gave positive reactions with nine sera, was assigned to the O78 serotype (three crosses in a dilution of 1:600), and the fourth strain, which gave positive reactions with seven sera, was assigned to serotype O111 (three crosses in a dilution of 1:3200). The results of serotyping show that the studied Escherichia coli strains have a complex antigenic structure.

Table 1. Escherichia strain serotype results in lamellar RA.

<table>
<thead>
<tr>
<th>Serums</th>
<th>Strains E.coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O26</td>
</tr>
<tr>
<td>O26</td>
<td>+</td>
</tr>
<tr>
<td>O55</td>
<td>-</td>
</tr>
<tr>
<td>O78</td>
<td>+</td>
</tr>
<tr>
<td>O111</td>
<td>-</td>
</tr>
<tr>
<td>O114</td>
<td>-</td>
</tr>
<tr>
<td>O119</td>
<td>+</td>
</tr>
<tr>
<td>O124</td>
<td>+</td>
</tr>
<tr>
<td>O125</td>
<td>+</td>
</tr>
<tr>
<td>O127</td>
<td>+</td>
</tr>
<tr>
<td>O128</td>
<td>+</td>
</tr>
<tr>
<td>O142</td>
<td>+</td>
</tr>
<tr>
<td>O143</td>
<td>+</td>
</tr>
<tr>
<td>O144</td>
<td>+</td>
</tr>
<tr>
<td>Total position reactions</td>
<td>17</td>
</tr>
</tbody>
</table>
Table 2. Frequency of isolation of Escherichia serotypes in birds in poultry farms of the Republic of Uzbekistan at different times

<table>
<thead>
<tr>
<th>Year</th>
<th>From whom are allocated</th>
<th>Serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>Chickens, hens</td>
<td>O2, O18, O26, O41, O55, O78, O109, O111, O142, O149</td>
</tr>
<tr>
<td>2010</td>
<td>Chickens, hens</td>
<td>O26, O55, O78, O111</td>
</tr>
<tr>
<td>2011</td>
<td>Chickens</td>
<td>O26, O41, O55, O78, O111</td>
</tr>
<tr>
<td>2012</td>
<td>Chickens</td>
<td>O26, O41, O55, O78, O111</td>
</tr>
</tbody>
</table>

As can be seen from the data in Table 2, the largest (10 serotypes) number of Escherichia strains: O2, O18, O26, O41, O55, O78, O111, O109, O142, O149 were isolated from poultry farms in the Republic of Uzbekistan in 2009; at the same time, in 2010, only 4 strains were isolated: O26, O55, O78, and O111; and in 2011 and 2012, respectively, 5 strains were isolated from poultry farms in the Republic of Uzbekistan, Consequently, O26, O41, O55, O78, and O111 are most often isolated from poultry farms in the Republic of Uzbekistan, which should be taken into account when preparing vaccines against avian escherichiosis.

The results of studying the biochemical properties of isolated Escherichia coli strains in poultry farms in the Republic of Uzbekistan are presented in Table 3.

Table 3. Results of studying the biochemical properties of isolated Escherichia coli strains in poultry farms in the Republic of Uzbekistan

<table>
<thead>
<tr>
<th>Strains</th>
<th>S A X A R A</th>
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<tbody>
<tr>
<td></td>
<td>lactose</td>
</tr>
<tr>
<td>O9</td>
<td>+ + + + -</td>
</tr>
<tr>
<td>O26</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>O41</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>O55</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>O78</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>O111</td>
<td>+ + + + -</td>
</tr>
</tbody>
</table>

Note: (K) - acid (G) - gas (+) - positively (-) - negative
Escherichia coli serotype O26 tested positive, i.e., it formed acid and released gas with lactose, glucose, maltose, dulcitol, mannitol, and sorbitol, gave a negative reaction with rhamnose, did not emit hydrogen sulfide, and did not split urea. The reaction with citrate and sodium malonate was positive. With indole, there was a positive reaction.

Escherichia coli serotype O41 tested positive, i.e., it formed acid and released gas with lactose, glucose, maltose, dulcitol, mannitol, rhamnose, and sorbitol, released hydrogen sulfide, and split urea. The reaction with sodium citrate and malonate was positive, and it was negative with indole.

The O55 serotype had biochemical properties similar to those of O41, except that in reaction with mannitol and sorbitol, it did not form acids, did not emit hydrogen sulfide, and did not break down urea. Reactions with citrate and sodium malonate were positive. The indole test was negative.

Serotype O78 had similar biochemical properties to O55 but did not break down urea.

Serotype O111 also had pronounced biochemical properties, but it had a negative reaction with maltose (did not form acid), dulcitol, and mannitol and did not form acid or indole with sorbitol.

The study of the cultural properties of Escherichia coli strains showed that they did not grow on Simmons' medium, did not ferment milk, did not liquefy gelatin, had a negative Voges-Proskauer reaction, and had samples with methylrot. All strains—isolates isolated by us on the poultry farms of the Republic were bacteria 1.53 and 0.5–0.8 microns in size, did not form spores or capsules, were motile, and stained negatively according to Gram. All of them were not demanding on nutrient media and, as a rule, grew well at pH 7.2–7.4.

On dense media, juicy gray-white colonies of medium (2–4 mm) or small (1–2 mm) size were formed. On liquid media, turbidity and a precipitate were given. Best of all, it grew at a temperature of +35–37°C.

On the Endo medium, E. coli formed colonies of a red color with a metallic sheen. The cultures possessed a wide range of enzymes: they formed indole on media, reduced nitrites and nitrates, fermented glucose, sucrose, lactose, maltose, xylose, and rhamnose to acid and gas, coagulated and peptonized milk, and on litmus milk caused redness.

The pathogenicity of the above strains was studied in chickens. Clinically healthy 2-week-old chickens were used for infection. They were infected intraperitoneally with a daily agar culture at a dose of 2 mlid. b.w. LD50/ml. Infected chickens showed general lethargy, loss of appetite, depression, mild fever, increased thirst, and gray-white liquid droppings. As a rule, all infected chickens died. The original strains were isolated from them.

In another experiment, we studied the characteristics of local Escherichia coli strains isolated from suffocating embryos, day-old chicks, young animals of various ages, and laying hens on a poultry farm.

During bacteriological examination of pathological material, 39 cultures of Escherichia coli were isolated. Their morphological, tinctorial, cultural, and biochemical properties were studied, and virulence was established for week-old egg-bearing chickens that were not immune to this disease. As a result, Escherichia coli cultures were isolated from suffocating embryos, 15-day-old chickens, and the follicles of sick laying hens. In Escherichia coli cultures isolated from pathological material, the following serotypes (O20, O26, O41, O55, O78, O111, and O119) were identified: To determine the virulence of the isolated strains, 10 chickens were infected with the isolates, which, after 3–4 days, showed a clinic of escherichiosis and died. During the autopsy of the dead birds, typical pathoanatomical changes in the parenchymal organs, characteristic of this disease, were found.
5 Discussion of the obtained results

Escherichiosis is one of the most common infections in birds of all ages. Among bacterial infections, escherichiosis is the most common and is pathogenic to chickens and adult birds. The main reasons for the spread of the disease are the high concentration of birds in a limited space, the variety of E. coli serovariants, the presence of many ways of infection transmission, opportunistic microflora circulating in the environment, and violations of veterinary and sanitary standards for keeping poultry.

The etiological structure of escherichiosis is diverse. Escherichia coli identification showed that more than 10 serovariants of the causative agent of escherichiosis are widespread in the Republic of Uzbekistan. The epizootic situation for avian escherichiosis in the Republic is mainly composed of such serovariants as O2, O18, O26, O41, O55, O78, O111, O109, O142, and O149. The percentage of these serovariants on average for 5 years was in the range of 6.0–19.8%. The data obtained by us basically coincide with the results of the studies of Borisenkov A.N. (2004), Bessarabova B.F. with co-authors (2007), Vengurenko L.A. (2009), Kapustina A.V. (2001), Radchuk T.N. (1990), and Plitov I.S. (2012). There are slight discrepancies in the percentage of individual serovars.

Clinical symptoms of the disease and pathological changes are of some importance in the diagnosis of escherichiosis. The study of the clinical course of escherichiosis in chickens and laying hens did not reveal any difference in the symptoms of the manifestation of the disease from those described by other scientists. In chickens, the disease often took an acute form. A common symptom of escherichiosis is a strong thirst, a rise in temperature by 1–1.5 °C, and an upset of the gastrointestinal tract with the appearance of foamy white diarrhea. To these signs in chickens were added the pallor of the comb and earrings and a sharp decrease in egg production. The characteristic changes in pathoanatomical autopsy were hyperemia of the intestinal mucosa and enlargement of the spleen and liver. In adult chickens, hepatization of the lungs, fibrinous plaque on the serous membranes of the intestines and internal organs, blockage of the oviduct, and sometimes peritonitis were noted. The same pathoanatomical features are described by Vinokurov V.Yu., Malysheva I.A. (2006), and Chernykh M.N. (2009).

Of the numerous representatives of the family, numbering more than 30 genera and more than 100 species, bacteria of the genera Salmonella, Escherichia, and Enterobacter take part in the pathology of birds (Tugarinov O.A., 1987; Sidorov M.A. et al., 1995; Smirnova L.I., 1996; Kapustin A.V., 2001; Artemyeva T.N., 2004; Salautin V.V., 2004; Panasenko A.S., 2008; Andersson Y., Jong B., 1996; Krysta H.R., 2006).

When studying the etiological structure of diseases of the digestive organs of birds caused by pathogenic Escherichia coli, the diagnosis was established on the basis of bacteriological studies, taking into account epizootological data, clinical signs of the disease, and pathomorphological changes. Differentiating signs of Escherichia coli were determined. For identification, the studied cultures of microorganisms were sown on differential media, followed by reseeding of isolated colonies to study the biological and enzymatic properties.

In a bacteriological study from a representative sample of poultry facilities, 132 cultures of microorganisms of the genus E. coli (O2, O18, O26, O41, O55, O78, O111, O109, O142, and O149) were isolated and identified. Research methods conducted by other foreign authors (Borisenkova A.N. (2004), Bessarabova B.F. et al., 2007)

6 Conclusion

As a result of a complex of diagnostic studies, a moderate spread (5–10%) of escherichiosis was found in the poultry farms of the Republic of Uzbekistan among chickens and hens.
Moreover, the disease was noted in the farms, both on the floor and in the cellular content. In chickens that died from escherichiosis, aged from several hours to 7–10 days, pathoanatomical changes characteristic of septic diseases were established. In older chickens, fibrin deposits were found on the pericardium and in the liver capsule. In adults, yolk peritonitis, catarrhal-fibrinous salpingitis, and atrophy of the ovary and oviduct were found.

The diagnosis of escherichiosis in birds in the conditions of poultry farms was made using bacteriological studies of smears of prints and the isolation of pure cultures of E. coli from parenchymal organs and bone marrow. The isolated Escherichia were bacteria without a capsule, 1.53 and 0.5–0.8 m in size, possessing motility, and stained negatively according to Gram. They were not demanding on nutrient media and, as a rule, grew well at pH 7.2–7.4.

On dense nutrient media, juicy, gray-white colonies of medium (2–4 mm) or small (1–2 mm) size were formed. On liquid media, turbidity and a precipitate were given. Red colonies with a metallic sheen formed on Endo's medium.

In the period 2009–2012, serotypes of Escherichia coli were most often identified in poultry farms as O26, O41, O55, O78, and O111. The isolated Escherichia coli had high enzymatic activity. All strains produced acid and gassed with lactose and glucose. Reactions with citrate and sodium malonate were positive. Five strains (O41, O78, O9, O26, and O55) out of six formed acids and evolved gases with maltose, rhamnose, mannitol, sorbitol, and dulcitol. Three (O41, O55, and O101) of the six strains did not form indole, and O26, O55, and O78 did not cleave urea. Two strains (O26 and O55) did not emit hydrogen sulfide. At the same time, strain O111 did not form acid with maltose, dulcitol, mannitol, or sorbitol and also did not release gases with dulcitol and mannitol.

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